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(54) Title: SERUM BIOMARKERS IN LUNG CANCER

MARKER ID	NO	FRACTION	MARKER ID	NO	FRACTION	MARKER ID	NO	FRACTION	MARKER ID	NO	FRACTION
MA1	2011	A	MA10	2020	A	MA19	2029	A	MA28	2038	A
MA2	2012	A	MA11	2021	A	MA20	2030	A	MA29	2039	A
MA3	2013	A	MA12	2022	A	MA21	2031	A	MA30	2040	A
MA4	2014	A	MA13	2023	A	MA22	2032	A	MA31	2041	A
MA5	2015	A	MA14	2024	A	MA23	2033	A	MA32	2042	A
MA6	2016	A	MA15	2025	A	MA24	2034	A	MA33	2043	A
MA7	2017	A	MA16	2026	A	MA25	2035	A	MA34	2044	A
MA8	2018	A	MA17	2027	A	MA26	2036	A	MA35	2045	A
MA9	2019	A	MA18	2028	A	MA27	2037	A	MA36	2046	A
MA10	2020	A	MA19	2029	A	MA28	2038	A	MA37	2047	A
MA11	2021	A	MA20	2030	A	MA29	2039	A	MA38	2048	A
MA12	2022	A	MA21	2031	A	MA30	2040	A	MA39	2049	A
MA13	2023	A	MA22	2032	A	MA31	2041	A	MA40	2050	A
MA14	2024	A	MA23	2033	A	MA32	2042	A	MA41	2051	A
MA15	2025	A	MA24	2034	A	MA33	2043	A	MA42	2052	A
MA16	2026	A	MA25	2035	A	MA34	2044	A	MA43	2053	A
MA17	2027	A	MA26	2036	A	MA35	2045	A	MA44	2054	A
MA18	2028	A	MA27	2037	A	MA36	2046	A	MA45	2055	A
MA19	2029	A	MA28	2038	A	MA37	2047	A	MA46	2056	A
MA20	2030	A	MA29	2039	A	MA38	2048	A	MA47	2057	A
MA21	2031	A	MA30	2040	A	MA39	2049	A	MA48	2058	A
MA22	2032	A	MA31	2041	A	MA40	2050	A	MA49	2059	A
MA23	2033	A	MA32	2042	A	MA41	2051	A	MA50	2060	A
MA24	2034	A	MA33	2043	A	MA42	2052	A	MA51	2061	A
MA25	2035	A	MA34	2044	A	MA43	2053	A	MA52	2062	A
MA26	2036	A	MA35	2045	A	MA44	2054	A	MA53	2063	A
MA27	2037	A	MA36	2046	A	MA45	2055	A	MA54	2064	A
MA28	2038	A	MA37	2047	A	MA46	2056	A	MA55	2065	A
MA29	2039	A	MA38	2048	A	MA47	2057	A	MA56	2066	A
MA30	2040	A	MA39	2049	A	MA48	2058	A	MA57	2067	A
MA31	2041	A	MA40	2050	A	MA49	2059	A	MA58	2068	A
MA32	2042	A	MA41	2051	A	MA50	2060	A	MA59	2069	A
MA33	2043	A	MA42	2052	A	MA51	2061	A	MA60	2070	A
MA34	2044	A	MA43	2053	A	MA52	2062	A	MA61	2071	A
MA35	2045	A	MA44	2054	A	MA53	2063	A	MA62	2072	A
MA36	2046	A	MA45	2055	A	MA54	2064	A	MA63	2073	A
MA37	2047	A	MA46	2056	A	MA55	2065	A	MA64	2074	A
MA38	2048	A	MA47	2057	A	MA56	2066	A	MA65	2075	A
MA39	2049	A	MA48	2058	A	MA57	2067	A	MA66	2076	A
MA40	2050	A	MA49	2059	A	MA58	2068	A	MA67	2077	A
MA41	2051	A	MA50	2060	A	MA59	2069	A	MA68	2078	A
MA42	2052	A	MA51	2061	A	MA60	2070	A	MA69	2079	A
MA43	2053	A	MA52	2062	A	MA61	2071	A	MA70	2080	A
MA44	2054	A	MA53	2063	A	MA62	2072	A	MA71	2081	A
MA45	2055	A	MA54	2064	A	MA63	2073	A	MA72	2082	A
MA46	2056	A	MA55	2065	A	MA64	2074	A	MA73	2083	A
MA47	2057	A	MA56	2066	A	MA65	2075	A	MA74	2084	A
MA48	2058	A	MA57	2067	A	MA66	2076	A	MA75	2085	A
MA49	2059	A	MA58	2068	A	MA67	2077	A	MA76	2086	A
MA50	2060	A	MA59	2069	A	MA68	2078	A	MA77	2087	A
MA51	2061	A	MA60	2070	A	MA69	2079	A	MA78	2088	A
MA52	2062	A	MA61	2071	A	MA70	2080	A	MA79	2089	A
MA53	2063	A	MA62	2072	A	MA71	2081	A	MA80	2090	A
MA54	2064	A	MA63	2073	A	MA72	2082	A	MA81	2091	A
MA55	2065	A	MA64	2074	A	MA73	2083	A	MA82	2092	A
MA56	2066	A	MA65	2075	A	MA74	2084	A	MA83	2093	A
MA57	2067	A	MA66	2076	A	MA75	2085	A	MA84	2094	A
MA58	2068	A	MA67	2077	A	MA76	2086	A	MA85	2095	A
MA59	2069	A	MA68	2078	A	MA77	2087	A	MA86	2096	A
MA60	2070	A	MA69	2079	A	MA78	2088	A	MA87	2097	A
MA61	2071	A	MA70	2080	A	MA79	2089	A	MA88	2098	A
MA62	2072	A	MA71	2081	A	MA80	2090	A	MA89	2099	A
MA63	2073	A	MA72	2082	A	MA81	2091	A	MA90	2100	A
MA64	2074	A	MA73	2083	A	MA82	2092	A	MA91	2101	A
MA65	2075	A	MA74	2084	A	MA83	2093	A	MA92	2102	A
MA66	2076	A	MA75	2085	A	MA84	2094	A	MA93	2103	A
MA67	2077	A	MA76	2086	A	MA85	2095	A	MA94	2104	A
MA68	2078	A	MA77	2087	A	MA86	2096	A	MA95	2105	A
MA69	2079	A	MA78	2088	A	MA87	2097	A	MA96	2106	A
MA70	2080	A	MA79	2089	A	MA88	2098	A	MA97	2107	A
MA71	2081	A	MA80	2090	A	MA89	2099	A	MA98	2108	A
MA72	2082	A	MA81	2091	A	MA90	2100	A	MA99	2109	A
MA73	2083	A	MA82	2092	A	MA91	2101	A	MA100	2110	A
MA74	2084	A	MA83	2093	A	MA92	2102	A	MA101	2111	A
MA75	2085	A	MA84	2094	A	MA93	2103	A	MA102	2112	A
MA76	2086	A	MA85	2095	A	MA94	2104	A	MA103	2113	A
MA77	2087	A	MA86	2096	A	MA95	2105	A	MA104	2114	A
MA78	2088	A	MA87	2097	A	MA96	2106	A	MA105	2115	A
MA79	2089	A	MA88	2098	A	MA97	2107	A	MA106	2116	A
MA80	2090	A	MA89	2099	A	MA98	2108	A	MA107	2117	A
MA81	2091	A	MA90	2100	A	MA99	2109	A	MA108	2118	A
MA82	2092	A	MA91	2101	A	MA100	2110	A	MA109	2119	A
MA83	2093	A	MA92	2102	A	MA101	2111	A	MA110	2120	A
MA84	2094	A	MA93	2103	A	MA102	2112	A	MA111	2121	A
MA85	2095	A	MA94	2104	A	MA103	2113	A	MA112	2122	A
MA86	2096	A	MA95	2105	A	MA104	2114	A	MA113	2123	A
MA87	2097	A	MA96	2106	A	MA105	2115	A	MA114	2124	A
MA88	2098	A	MA97	2107	A	MA106	2116	A	MA115	2125	A
MA89	2099	A	MA98	2108	A	MA107	2117	A	MA116	2126	A
MA90	2100	A	MA99	2109	A	MA108	2118	A	MA117	2127	A
MA91	2101	A	MA100	2110	A	MA109	2119	A	MA118	2128	A
MA92	2102	A	MA101	2111	A	MA110	2120	A	MA119	2129	A
MA93	2103	A	MA102	2112	A	MA111	2121	A	MA120	2130	A
MA94	2104	A	MA103	2113	A	MA112	2122	A	MA121	2131	A
MA95	2105	A	MA104	2114	A	MA113	2123	A	MA122	2132	A
MA96	2106	A	MA105	2115	A	MA114	2124	A	MA123	2133	A
MA97	2107	A	MA106	2116	A	MA115	2125	A	MA124	2134	A
MA98	2108	A	MA107	2117	A	MA116	2126	A	MA125	2135	A
MA99	2109	A	MA108	2118	A	MA117	2127	A	MA126	2136	A
MA100	2110	A	MA109	2119	A	MA118	2128	A	MA127	2137	A
MA101	2111	A	MA110	2120	A	MA119	2129	A	MA128	2138	A
MA102	2112	A	MA111	2121	A	MA120	2130	A	MA129	2139	A
MA103	2113	A	MA112	2122	A	MA121	2131	A	MA130	2140	A
MA104	2114	A	MA113	2123	A	MA122	2132	A	MA131	2141	A
MA105	2115	A	MA114	2124	A	MA123	2133	A	MA132	2142	A
MA106	2116	A	MA115	2125	A	MA124	2134	A	MA133	2143	A
MA107	2117	A	MA116	2126	A	MA125	2135	A	MA134	2144	A
MA108	2118	A	MA117	2127	A	MA126	2136	A	MA135	2145	A
MA109	2119	A	MA118	2128	A	MA127	2137	A	MA136	2146	A
MA110	2120	A	MA119	2129	A	MA128	2138	A	MA137	2147	A
MA111	2121	A	MA120	2130	A	MA129	2139	A	MA138	2148	A
MA112	2122	A	MA121	2131	A	MA130	2140	A	MA139	2149	A
MA113	2123	A	MA122	2132	A	MA131	2141	A	MA140	2150	A
MA114	2124	A	MA123	2133	A	MA132	2142	A	MA141	2151	A

SERUM BIOMARKERS IN LUNG CANCER

BACKGROUND OF THE INVENTION

[0001] The present invention relates generally to the field of serum biomarkers in lung carcinoma. More particularly, the invention relates to serum biomarkers that can distinguish lung cancer from normal.

[0002] Lung cancer is the leading cause of cancer death worldwide, resulting in 130,000 deaths per year in the United States. The mortality rate from lung cancer is greater than the combined mortality from breast, prostate and colorectal cancers. On the basis of morphology, lung cancer can be broadly classified into four main categories namely, adenocarcinoma, squamous cell carcinoma, large cell undifferentiated carcinoma and small cell carcinoma. In Hong Kong from 1990 to 1996, the proportions for adenocarcinoma, squamous cell carcinoma, large cell undifferentiated carcinoma and small cell carcinoma are 43.5%, 27.5%, 4.7% and 10.3% respectively. Both squamous cell carcinoma and small cell carcinoma are strongly associated with a smoking history.

[0003] Adenocarcinoma, squamous cell carcinoma, and large cell undifferentiated carcinoma are usually referred as "non-small cell carcinoma." They are relatively chemo-resistant, and hence the mainstay of treatment is surgery. By contrast, small cell carcinoma has a higher propensity for distant metastases and is mainly treated by chemotherapy.

[0004] Biopsy can be used to diagnose lung cancer, but it is an invasive procedure and, therefore, less than desirable. Other diagnostic methods for lung cancer include ultrasound and computed tomography (CT) scan.

[0005] It would be highly desirable to have a biomarker or combination of biomarkers capable of distinguishing between lung cancer and normal cells. In addition, a simple test could aid in tracking treatment progress and even identify molecular targets for therapy. The literature on lung cancer diagnosis has not disclosed heretofore such a biomarker or combination of biomarkers, however.

SUMMARY OF THE INVENTION

[0006] In accordance with the present invention, biomarkers and combinations of biomarkers are used to identify lung cancer. The method successfully distinguishes between lung cancer and normal states, and can be used to identify the particular type of lung cancer. In one embodiment, a method for qualifying lung carcinoma status in a subject (e.g., a patient) comprises analyzing a biological sample from the subject for one or more of the top 50 biomarkers as shown in Figure 2 or Figures 4A and 4B.

Thus, to assess overall lung cancer risk versus normal, a biomarker is selected from the group consisting of

(A) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-178, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268, or

(B) WM-61, WM-447, WM-448, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310.

[0007] wherein the biomarker is differentially present in samples of a subject with lung cancer and a so-called "normal" subject that is free of lung cancer.

[0008] More preferably, one or more of the top 15 biomarkers as shown in Figure 2 or Figures 4A and 4B is used to qualify lung cancer status. Thus, for assessing overall lung cancer status versus normal, the protein is selected from the group consisting of

(A) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-471, IM-510, IM-544, IM-474, and IM-155, or

(B) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70.

[0009] Still more preferably, one or more of the top 5 biomarkers as shown in Figure 2 or Figures 4A and 4B is used to qualify lung cancer status. In this instance, for overall lung cancer status versus normal, the biomarker is selected from the group consisting of

(A) IM-522, IM-273, IM-520, IM-519, and IM-454, or

(B) WM-61, WM-447, WM-446, WM-133, and WM-119.

[0010] In one embodiment, the method measures a plurality of biomarkers. The plurality of biomarkers can be measured simultaneously.

[0011] Biomarkers that, by themselves, are able to identify lung cancer include the WM-446 and WM-447 protein biomarkers, and these are particularly preferred.

[0012] The present invention also provides a method for qualifying lung cancer status in a subject (e.g., a patient), comprising (A) providing a spectrum generated by subjecting a biological sample from said subject to mass spectroscopic analysis that includes profiling on a chemically-derivatized affinity surface, and (B) putting the spectrum through pattern-recognition analysis that is keyed to at least one peak selected from the top 50 biomarkers as shown in Figure 2 or Figures 4A and 4B.

Thus, for qualifying overall lung cancer status, the biomarker is selected from the, group consisting of

(I) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-369, IM-436, IM-106, IM-465, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-

266 or

(B) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-282, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310.

[0013] For assessing the overall lung cancer status, the pattern-recognition analysis may, for example, be paired to a pair of peaks selected from the group consisting of (A) IM-266 and IM-474, IM-266 and IM-38, IM-266 and IM-454, IM-266 and IM-522, IM-266 and IM-544, IM-266 and IM-471, IM-474 and IM-151, IM-474 and IM-156, IM-474 and IM-544, IM-474 and IM-38, IM-522 and IM-507, IM-522 and IM-156, and IM-522 and IM-440;

or

(B) WM-447 and WM-59, WM-447 and WM-19, WM-447 and WM-118, WM-447 and WM-473, WM-19 and WM-59, WM-19 and WM-473, WM-19 and WM-369, WM-61 and WM-154, WM-61 and WM-369, WM-118 and WM-59 and WM-282 and WM-127.

[0014] More preferably, for assessing overall lung cancer status, the pattern-recognition analysis is keyed to a pair of peaks selected from the group consisting of (A) IM-266 and IM-474, IM-266 and IM-544, and IM-156 and IM-522;

or

(B) WM-447 and WM-59, WM-447 and WM-19, and WM-19 and WM-59.

[0015] Alternatively, the pattern-recognition analysis for assessing overall lung cancer status may be keyed to a triple of peaks selected from the group consisting of (A) IM-266, IM-454 and IM-474; and IM-266, IM-474 and IM-544;

or

(B) WM-447, WM-19 and WM-473.

[0016] In other embodiments, the pattern-recognition analysis may be keyed to a combination of more than three peaks, more particularly to a combination of 4, 5 or 6 peaks, where the combination is selected from among the combinations shown in Tables 1 and 2 herein.

[0017] In each case, the biomarker is differentially present in samples of a subject with lung cancer and a normal subject.

[0018] The invention also contemplates a kit for detecting and diagnosing lung cancer, thereby to assess lung cancer status. Kits within the invention comprise, for example, (i) an adsorbent attached to a substrate that retains one or more of the biomarkers shown in Figure 2 or Figures 4A and 4B, and (ii) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent. An inventive kit may further comprise a washing solution and/or instructions for making a washing solution. The kits may include more than one type of adsorbent, each present on a different substrate, e.g., on a WCC and IMAC biochip. In addition, the kits may comprise one or more containers with biomarker samples, to be used as standard(s) for calibration. The substrate comprising the adsorbent may be designed to engage a probe interface and, hence, function as a probe in gas phase ion spectrometry, preferably mass spectrometry. Alternatively, the kit may further comprise a second substrate adapted to engage the probe interface, on which the substrate comprising the adsorbent is mounted.

[0019] The method and kit according to the invention produce an article of manufacture in which one or more biomarkers according to the invention are bound to an adsorbent, optionally contacted with a matrix or energy absorbing molecule.

[0020] The present invention also provides software for qualifying lung carcinoma status in a subject, comprising an algorithm for analyzing data extracted from a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, wherein said data relates to one or more biomarkers according to the invention. In one embodiment, the algorithm carries out a pattern-recognition analysis that is keyed to data relating to at least one of the biomarkers. In another embodiment, the algorithm comprises classification tree analysis that is keyed to data relating to at least one of the biomarkers. In yet another embodiment, the algorithm

comprises an artificial neural network analysis that is keyed to data relating to at least one of the biomarkers.

[0021] In certain embodiments, the present invention provides methods and kits that use serum amyloid A protein or a fragment thereof to qualify lung carcinoma status in a subject. In one of these embodiments, the serum amyloid A biomarker has an apparent molecular weight of about 2803, 3168, 3277, 3552, 3897, 4300, 4490, 4655, 5927, 6874, 7776, 7941, 8152, 8952, 9233, 10300, 10866, or 10851 Daltons. In another embodiment, the serum amyloid A biomarker has an apparent molecular weight of about 3168, 3277, 3552, 3897, 4300, 4490, 4655, 7776, 7941, 8152, 8952, or 10851 Daltons. In yet another embodiment, the serum amyloid A biomarker has an apparent molecular weight of about 11.5 to 11.7 kD.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Figures 1A-1D show all biomarkers identified with a Cu(II) IMAC3 ProteinChip® array format.

[0023] Figure 2 shows the top 50 biomarkers identified with a Cu(II) IMAC3 ProteinChip® array format.

[0024] Figures 3A-3O show all biomarkers identified with a WCC ProteinChip® array format.

[0025] Figures 4A and 4B show the top 50 biomarkers identified with a WCC ProteinChip® array format.

[0026] Figure 5 shows fragments of serum amyloid A (SAA) that are biomarkers according to the present invention.

[0027] Figure 6 shows identification of SAA biomarkers with an anti-SAA antibody.

[0028] Figures 7-16 are spectra from WCC chips in which all of the top 15 WCC marker peaks are labeled, along with various other peaks from among the top 50 WCC peaks. Red shows spectra from lung cancer patients and gray shows normals.

[0029] Figures 17-28 are spectra from IMAC chips in which all of the top 15 WCC marker peaks are labeled, along with various other peaks from among the top 50 IMAC peaks. Blue shows spectra from lung cancer patients and gray shows normals.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0030] In accordance with the present invention, a series of biomarkers associated with lung cancer has been discovered. In the present context, a biomarker is an organic biomolecule, particularly a polypeptide or protein, which is differentially present in a sample taken from a subject having lung cancer as compared to a comparable sample taken from a normal subject. A biomarker also may be differentially present in a sample taken from a subject with one type of lung cancer, e.g., small cell carcinoma, as compared to a comparable sample taken from a subject with a different type of lung cancer, e.g., adenocarcinoma or squamous cell carcinoma, or differentially present at different stages of a type of lung cancer. A biomarker is differentially present in samples taken from two groups of subjects if it is present at an elevated level or a decreased level in samples of the first group as compared to samples of the second group. More particularly, a biomarker is a polypeptide that is characterized by an apparent molecular weight, as determined by mass spectrometry, and that is present in samples from lung cancer subjects in an elevated or decreased level, as compared to subjects that do not have lung cancer. A biomarker is differentially present between two sets of samples if the amount of the biomarker in one sample set differs in a statistically significant way ($p < 0.01$) from the amount of biomarker in the other sample set.

[0031] The biomarkers of the invention can be used to assess lung cancer status in a subject. For example, they are capable of identifying lung cancer and successfully distinguishing it from normal subjects, thereby providing a way of diagnosing the presence or absence of lung cancer, including the presence or absence of a particular kind of lung cancer. In addition, the biomarkers are useful in assessing the risk of developing lung cancer, in staging of lung cancer and in assessing the effectiveness of treatment. Thus, "lung cancer status" in the context of the present invention includes, *inter alia*, the presence or absence of disease, the risk of developing disease, the stage of the disease, and the effectiveness of treatment of disease. Based on this status, further procedures may be indicated, including additional diagnostic tests or therapeutic procedures or regimens, such as endoscopy, biopsy, surgery, chemotherapy, immunotherapy, and radiation therapy.

[0032] In some instances, a single biomarker is capable of identifying lung cancer with a sensitivity or specificity of at least 85%, whereas, in other instances, a combination or plurality of biomarkers is used to obtain a sensitivity or specificity of at least 85%. The biomarkers and combinations of biomarkers thus can be used to qualify lung cancer status in a subject or patient.

[0033] The biomarkers according to the invention are present in serum. The biological sample used according to the present invention, however, need not be a serum sample. Thus, a biological sample for qualifying lung cancer status may be a serum, plasma or blood sample, although serum samples are preferred.

[0034] All of the biomarkers are characterized by molecular weight. A list of all the biomarkers obtained with the Cu(II) IMAC3 ProteinChip® array (Ciphergen Biosystems, Inc., Fremont, California, USA) is provided in Figures 1A-1D, and Figure 2 lists the top 50 biomarkers that distinguish between lung cancer and normal subjects that are identified by Cu(II) IMAC3 protocol described herein. Figures 3A-3O comprise a list of all the biomarkers obtained with the WCX2 ProteinChip® array, and Figures 4A and 4B comprise a ranking of the top 50 biomarkers that distinguish between (i) lung cancer and normal subjects, (ii) subjects with each of four types of lung cancer and normal subjects, and (iii) two types of lung cancer, e.g., adenocarcinoma versus squamous cell carcinoma, as identified by WCX2 protocol described herein.

[0035] The top 50 biomarkers were determined by decision tree analysis using Biomarker Patterns™ software from Ciphergen Biosystems, Inc. Biomarkers other than those within the top 50 also are useful in distinguishing between subjects with lung cancer and normal subjects and may, in particular, appear in decision trees with multiple nodes. In preferred embodiments, one or more of the top 15 biomarkers are used, and in even more preferred embodiments, one or more of the top 5 biomarkers are used.

[0036] In each of Figures 1A-1D and 3A-3O, the number in the first column is the biomarker identifier. Thus, the first row in Figures 1A-1D relates to biomarker IM-1, the second row relates to biomarker IM-2, and so forth ("IM-" denoting biomarkers identified with the IMAC chip). Similarly, the first row in Figures 3A-3O relates to

biomarker WM-1 and the second row relates to biomarker WM-2 ("WM" denoting biomarkers identified with the WCX2 chip). The number in the second column in Figures 1A-1D is the apparent molecular weight of the biomarker in daltons, as determined by mass spectrometry. In Figures 3A-3O, the apparent molecular weights for the biomarkers identified in the first column are reported in columns 3 through 11. The letter in the second column of Figures 1A-1D and the third column of Figures 3A-3O denotes the fraction in which the biomarker elutes in the protocol described herein; that is, biomarkers with an "A" elute in the first fraction, biomarkers with a "B" elute in the second fraction, and so forth. The fraction in which the biomarker elutes correlates with its pI, which biomarkers eluting at higher pH having a higher pI, and biomarkers eluting at lower pH having a lower pI.

[0037] Presenting the mass and affinity characteristics of a given biomarker within the invention, as in this description, characterizes that biomarker so as allow one to obtain and measure it, in accordance with the teachings herein. If desired, any of the biomarkers can be sequenced, in order to obtain an amino acid sequence, but this is not required to practice the present invention.

[0038] For example, a biomarker can be peptide mapped with a number of enzymes, such as trypsin and V8 protease, and the molecular weights of the digestion fragments can be used to search databases for sequences that match the molecular weights of the digestion fragments generated by the various enzymes. Alternatively, if the biomarkers are not proteins included in known databases, degenerate probes can be made based on the N-terminal amino acid sequence of the biomarker, which then are used to screen a genomic or cDNA library created from a sample from which the biomarker was initially detected. The positive clones can be identified, amplified, and their recombinant DNA sequences can be subcloned using techniques which are well known. Finally, protein biomarkers can be sequenced using protein ladder sequencing. Protein ladders can be generated by fragmenting the molecules and subjecting fragments to enzymatic digestion or other methods that sequentially remove a single amino acid from the end of the fragment. The ladder is then analyzed by mass spectrometry. The difference in masses of the ladder fragments identifies the amino acid removed from the end of the molecule.

[0039] Several biomarkers identified in accordance with the teachings of the present invention fit to serum amyloid A (SAA) or to a fragment of SAA. SAA is a well-known acute phase inflammatory marker. A number of the SAA biomarkers are identified in Figure 3 by both molecular mass and amino acid sequence. Most of these markers bound anti-SAA antibodies, as shown in Figure 6. The intact mass of SAA is 11.5 to 11.7 kD, and these biomarkers also have been identified by the present methodology. Fragments preferably have a molecular mass of at least about 200 Daltons, more preferably at least about 500 Daltons. In even more preferred embodiments, fragments have a molecular mass of at least about 800 Daltons, and most preferably at least about 1 KiloDalton.

[0040] In one embodiment, the fragments of SAA include a sequence of amino acids that is recognized by an epitope of an anti-SAA antibody. One way of identifying suitable fragments for use in the present invention is to enzymatically digest SAA and test the resulting fragments for the ability to bind to an anti-SAA antibody. Fragments that bind anti-SAA antibody can be sequenced using techniques well-known in the art, although the sequence of the fragment is not needed to practice the invention. In order to practice the invention with a fragment from the enzymatic digest that is identified as binding anti-SAA antibody, all that is required is to subject to the fragment to mass spectrometry to determine its mass.

[0041] The serum biomarkers according to the present invention were identified by comparing mass spectra of samples derived from sera from two groups of newly-diagnosed subjects, subjects with lung cancer and normal subjects. The subjects were diagnosed according to standard clinical criteria. Lung cancer subjects were histologically confirmed, and subjects without lung cancer were followed for at least 18 months following serum collection for any sign of lung cancer, to exclude subjects with asymptomatic lung cancer.

[0042] Sera from each group of subjects was collected, and fractionated with Q Ceramic HyperDF ion exchange resin (Bioseptra SA, France) into six fractions which eluted at different pH. Fraction A comprised the flow through plus pH 9 eluant, Fraction B comprised the pH 7 eluant, Fraction C comprised the pH 5 eluant, Fraction D comprised the pH 4 eluant, Fraction E comprised the pH 3 eluant, and Fraction F

comprised isopropyl alcohol/acetronitrile TFA eluant. Fractions A through F are identified on Figures 7-28 as Fractions 1 through 6, respectively.

[0043] Each fraction was diluted and applied to a ProteinChip® array, either a Cu(II) IMAC3 or WCX2 chip array. Both of these chip arrays are produced by Ciphergen Biosystems, Inc. (Fremont, CA).

[0044] The Cu(II) IMAC3 is an "immobilized metal affinity-capture" chip, with a nitrilotriacetic acid surface for high-capacity copper binding and subsequent affinity capture of proteins with metal binding residues. Imidazole may be used in binding and washing solutions to moderate protein binding, including binding of non-specific proteins. Increasing the concentration of imidazole in the washing buffers reduces the binding of the target proteins. It is produced by photopolymerizing 5-methylacrylamido-2-(N,N-bis(carboxymethylamino)pentanoic acid (7.5 wt%) and N,N'-methylenebisacrylamide (0.4 wt%) using (-) riboflavin (0.02 wt%) as a photoinitiator. The monomer solution is deposited onto the chip substrate and irradiated to photopolymerize. The chip then is activated with Cu(II).

[0045] The WCX2 is a weak cation exchange array with a carboxylate surface to bind cationic proteins. The negatively charged carboxylate groups on the surface of the WCX2 chip interact with the positive charges exposed on the target proteins. The binding of the target proteins is reduced by increasing the concentration of salt or by increasing the pH of the washing buffers.

[0046] Following application of the eluant fraction, the chips were incubated to allow the polypeptides in the eluant to bind to the sites on the chip by an affinity interaction. After incubation, each chip array was washed to remove polypeptides that bind non-specifically and buffer contaminants. That chip then was dried, and an energy absorbing molecule or matrix was applied to it, to facilitate desorption and ionization in a mass spectrometer.

[0047] In the mass spectrometer, retained polypeptides were desorbed from the chip array by laser desorption and ionization in a ProteinChip® Reader, which is integrated with ProteinChip® Software and a personal computer to analyze proteins captured on chip arrays. The ion optic and laser optic technologies in the ProteinChip® Reader detects proteins ranging from small peptides of less than 1000 Da up to proteins of

300 kilodaltons or more, and calculates the mass based on time-of-flight. Ionized polypeptides were detected and their mass accurately determined by this Time-of-Flight (TOF) Mass Spectrometry.

[0048] The mass spectra obtained for each group were subjected to scatter plot analysis, to eliminate run-to-run variation. Protein clusters on the scatter plot that had the same pattern for both lung cancer and normal subjects, i.e., protein clusters that were either elevated in both groups of subjects or depressed in both groups of subjects, were eliminated as potential biomarkers. The remaining polypeptides were further analyzed for their ability to accurately identify subjects with lung cancer. Because the molecular weights were derived from scatter plot analysis, and because of limits on the ability of mass spectrometry to resolve molecular weights, the "absolute" molecular weight values given in Figures 1A-1D and 3A-3O actually represent approximate molecular weights.

[0049] The biomarkers of this invention are characterized by their mass-to-charge ratio as determined by mass spectrometry. The mass-to-charge ratio of each biomarker is provided in Figures 1A-1D and 3A-3O. For example, IM-1 in Figure 1A has a measured mass-to-charge ratio of 2011. The mass-to-charge ratios were determined from mass spectra generated on a Ciphergen Biosystems, Inc. PBS II mass spectrometer. This instrument has a mass accuracy of about +/- 0.15 percent.

Additionally, the instrument has a mass resolution of about 400 to 1000 m/dm, where m is mass and dm is the mass spectral peak width at 0.5 peak height. The mass-to-charge ratio of the biomarkers was determined using Biomarker Wizard™ software (Ciphergen Biosystems). Biomarker Wizard assigns a mass-to-charge ratio to a biomarker by clustering the mass-to-charge ratios of the same peaks from all the spectra analyzed, as determined by the PBSII, taking the maximum and minimum mass-to-charge-ratio in the cluster, and dividing by two. Accordingly, the masses provided reflect these specifications.

[0050] The biomarkers of this invention are further characterized by the shape of their spectral peak in time-of-flight mass spectrometry. Mass spectra showing peaks representing the biomarkers are presented in Figures 7-28. The biomarker identifier numbers from Figures 2 and 4A-4B, respectively, are shown next to the peak, along

with their rank, which is indicated in parentheses below the biomarker identifier number.

[0051] The biomarkers of this invention are further characterized by their binding properties on chromatographic surfaces. Most of the biomarkers bind to IMAC (Cu) or WCX adsorbents (e.g., the Cphergent® IMAC (Cu) or WCX ProteinChip® arrays) after washing as described herein.

[0052] Thus, a given molecular weight for a biomarker herein should be interpreted as the midpoint of a molecular-weight range. The accuracy of the mass spectrometer is $\pm 0.15\%$, and the actual molecular weight for a biomarker is therefore the value given, $\pm 0.15\%$. For example, the actual molecular weight for biomarker IM-273 is $11705 \pm 0.15\%$, or between 11687 and 11722. Often, the range surrounding the "absolute" value given in the figure is no more than ± 5 daltons (2006 to 2016 for IM-1), generally no more than ± 3 daltons (2008 to 2014 for IM-1), and often as small as ± 1 dalton (2010 to 2012 daltons for IM-1).

[0053] CART® (Salford Systems, San Diego, CA), a classification and regression tree software, was used to determine whether a potential biomarker had predictive value in assessing lung cancer. A software macro randomly selected a subset of 15% of the peaks from Figures 1A-1D or Figures 3A-3O. The peaks and peak heights from each sample were provided to the CART® software for analysis. The software performed an iterative analysis until a single decision tree was generated that was capable of distinguishing between cancerous and non-cancerous. Each node in the resulting decision tree sorted based on the peak height of a single biomarker. A tree may contain any number of nodes, but generally contains from 1 to 6 nodes. From a practical standpoint in a commercial diagnostic test, a decision tree with fewer nodes is preferred. A total of 2000 decision trees, each based on a different 15% subset of the peaks from Figures 1A-1D or Figures 3A-3O, were generated.

[0054] The CART® software assigned a score to each biomarker in the subset based on its relative importance. A score of 100 is very high and a score of 0 is very low. The CART® software also determined the sensitivity and specificity of each decision tree.

[0055] The data generated by the decision tree analysis was subjected to further analysis. The biomarkers were ranked based on their average scores, which were determined by adding up a biomarker's scores for each decision tree in which it appeared, and dividing by the total number of decision trees in which the biomarker appeared. Approximately 500 of the potential biomarkers showed up in at least one tree, and most of the biomarkers showed up in about 150 to 400 of the two thousand trees. The top 50 biomarkers for the IMAC and WCX chip arrays as determined by this method are shown in Figures 2 and 4A-4B, respectively.

[0056] All of the trees having sensitivities and specificities greater than 85% also were identified. All trees capable of distinguishing lung cancer from normal and having from 1 to 6 nodes that meet the 85/85 criterion are shown in Tables 1 and 2.

TABLE 1. Decision trees with IMAC Biomarkers.

2 Nodes			
474	151		
474	156		
522	507		2 trees
522	440		2 trees
3 Nodes			
266	454	474	
474	156	153	
474	40	156	
520	276	113	
520	265	401	
522	151	474	
522	478	153	
522	156	474	
4 Nodes			
148	521	508	251

266	544	474	483	
266	157	126	420	
266	544	474	482	
266	471	474	38	
266	544	474	38	
266	514	471	203	
522	58	266	474	
5 Nodes				
266	544	473	151	437
266	454	474	153	264
273	143	544	401	199

TABLE 2. Decision Trees with WCX Biomarkers.

1 Node				
446				
447				
2 Nodes				
282	127			
3 Nodes				
61	16	27		
61	119	154		
61	120	154		
61	369	184		
61	184	129		
61	19	282		
133	282	319		
282	59	218		
282	111	65		

448	19	16		
4 Nodes				
61	369	282	184	
61	48	203	3	
448	369	111	67	
446	466	58	120	
446	19	59	113	
446	282	19	47	
447	118	59	417	
447	118	59	473	
447	65	59	275	
447	19	59	282	
447	369	59	206	
447	19	59	253	
447	19	47	70	
5 Nodes				
61	369	128	184	197
61	17	425	366	341
133	139	363	216	273
282	133	48	19	253
369	310	19	109	384
448	282	15	319	88
447	19	71	473	31
447	19	17	473	438
447	47	31	365	59
6 Nodes				
369	366	182	471	19
				439

[0057] Each of the biomarker combinations of Tables 1 and 2 are preferred combinations for distinguishing lung cancer subjects from normal subjects in accordance with the present invention.

[0058] All biomarkers that appeared in at least two of the trees that met the 85/85 criterion were identified. For these biomarkers, Tables 3 and 4 provide the number of times the biomarker occurred in a trees that met the criterion, as well as the ranking of that biomarker on the top 50 lists of Figures 2 and 4A-4B.

TABLE 3. Correlation of IMAC biomarker decision tree frequencies and ranking.

Peak	# times	Rank
286	9	9
522	8	1
474	4	14
520	2	3
148	1	8
273	1	2

TABLE 4. Correlation of WCX biomarker decision tree frequencies and ranking.

Peak	# times	Rank
447	11	2
61	10	1
446	7	3
282	4	9
369	2	8
133	2	4

[0059] Biomarkers that occurred frequently in the highly discriminatory trees occurred among the top 50 ranked biomarkers, and typically had a top 10 ranking. In addition, certain pairs of biomarkers reappear, e.g., WM-447 and WM-59, WM-447 and WM-19, WM-19 and WM-59, IM-266 and IM-474, IM-266 and IM-38, IM-266 and IM-454, IM-522 and IM-266. There also are repeats among triplets of biomarkers, such as IM-266, IM-266 and IM-38, and WM-447, WM-19 and WM-473. Other repeating pairs and trios of biomarkers can be seen in Tables 3 and 4, and are preferred.

[0060] Biomarkers and combinations of biomarkers identified in accordance with the present description may be used to qualify lung cancer status in a subject. In particular, a biomarker or combination of biomarkers can be used to distinguish lung cancer patients from normal patients with a high degree of specificity or sensitivity, i.e., greater than at least 85%, preferably greater than at least 90%, and more preferably greater than 95%.

[0061] According to one aspect of the invention, therefore, the detection of biomarkers for diagnosis of lung cancer status entails contacting a sample from a subject with a substrate, e.g., a SELDI probe, having an adsorbent thereon, under conditions that allow binding between the biomarker and the adsorbent, and then detecting the biomarker bound to the adsorbent by gas phase ion spectrometry, for example, mass spectrometry. Other detection paradigms that can be employed to this end include optical methods, electrochemical methods (voltammetry and amperometry techniques), atomic force microscopy, and radio frequency methods, e.g., multipolar resonance spectroscopy. Illustrative of optical methods, in addition to microscopy, both confocal and non-confocal, are detection of fluorescence, luminescence, chemiluminescence, absorbance, reflectance, transmittance, and birefringence or refractive index (e.g., surface plasmon resonance, ellipsometry, a resonant mirror method, a grating coupler waveguide method or interferometry).

[0062] In one aspect, the markers of this invention are detected by gas phase ion spectrometry, which refers to the use of a gas phase ion spectrometer to detect gas phase ions. A gas phase ion spectrometer is an apparatus that detects gas phase ions. Gas phase ion spectrometers include an ion source that supplies gas phase ions. Gas

phase ion spectrometers include, for example, mass spectrometers, ion mobility spectrometers, and total ion current measuring devices.

[0063] "Mass spectrometer" refers to a gas phase ion spectrometer that measures a parameter which can be translated into mass-to-charge ratios of gas phase ions. Mass spectrometers generally include an ion source and a mass analyzer. Examples of mass spectrometers are time-of-flight, magnetic sector, quadrupole filter, ion trap, ion cyclotron resonance, electrostatic sector analyzer and hybrids of these. "Mass spectrometry" refers to the use of a mass spectrometer to detect gas phase ions. "Laser desorption mass spectrometer" refers to a mass spectrometer which uses laser as a means to desorb, volatilize, and ionize an analyte.

[0064] "Mass analyzer" refers to a sub-assembly of a mass spectrometer that comprises means for measuring a parameter which can be translated into mass-to-charge ratios of gas phase ions. In a time-of-flight mass spectrometer the mass analyzer comprises an ion optic assembly, a flight tube and an ion detector.

[0065] "Ion source" refers to a sub-assembly of a gas phase ion spectrometer that provides gas phase ions. In one embodiment, the ion source provides ions through a desorption/ionization process. Such embodiments generally comprise a probe interface that positionally engages a probe in an interrogatable relationship to a source of ionizing energy (e.g., a laser desorption/ionization source) and in concurrent communication at atmospheric or subatmospheric pressure with a detector of a gas phase ion spectrometer.

[0066] Forms of ionizing energy for desorbing/ionizing an analyte from a solid phase include, for example: (1) laser energy; (2) fast atoms (used in fast atom bombardment); (3) high energy particles generated via beta decay of radionuclides (used in plasma desorption); and (4) primary ions generating secondary ions (used in secondary ion mass spectrometry). The preferred form of ionizing energy for solid phase analytes is a laser (used in laser desorption/ionization), in particular, nitrogen lasers, Nd-Yag lasers and other pulsed laser sources. "Fluence" refers to the laser energy delivered per unit area of interrogated image. Typically, a sample is placed on the surface of a probe, the probe is engaged with the probe interface and the probe

surface is struck with the ionizing energy. The energy desorbs analyte molecules from the surface into the gas phase and ionizes them.

[0067] Other forms of ionizing energy for analytes include, for example: (1) electrons which ionize gas phase neutrals; (2) strong electric field to induce ionization from gas phase, solid phase, or liquid phase neutrals; and (3) a source that applies a combination of ionization particles or electric fields with neutral chemicals to induce chemical ionization of solid phase, gas phase, and liquid phase neutrals.

[0068] A preferred mass spectrometric technique for use in the invention is Surface Enhanced Laser Desorption and Ionization (SELDI), as described, for example, in U.S. patents No. 5,719,060 and No. 6,225,047, both to Hutchens and Yip, in which the surface of a probe that presents the analyte (here, one or more of the biomarkers) to the energy source plays an active role in desorption/ionization of analyte molecules. In this context, "probe" refers to a device adapted to engage a probe interface and to present an analyte to ionizing energy for ionization and introduction into a gas phase ion spectrometer, such as a mass spectrometer. A probe typically includes a solid substrate, either flexible or rigid, that has a sample-presenting surface, on which an analyte is presented to the source of ionizing energy.

[0069] One version of SELDI, called Surface-Enhanced Affinity Capture" or "SEAC," involves the use of probes comprised of a chemically selective surface ("SELDI probe"). A "chemically selective surface" is one to which is bound either the adsorbent, also called a "binding moiety" or "capture reagent," or a reactive moiety that is capable of binding a capture reagent, e.g., through a reaction forming a covalent or coordinate covalent bond.

[0070] The phrase "reactive moiety" here denotes a chemical moiety that is capable of binding a capture reagent. Epoxide and carbodimidazole are useful reactive moieties to covalently bind polypeptide capture reagents such as antibodies or cellular receptors. Nitroloacetic acid and iminodiacetic acid are useful reactive moieties that function as chelating agents to bind metal ions that interact non-covalently with histidine containing peptides. A "reactive surface" is a surface to which a reactive moiety is bound. An "adsorbent" or "capture reagent" can be any material capable of

binding a biomarker of the invention. Suitable adsorbents for use in SELDI,

according to the invention, are described in U.S. patent No. 6,225,047, *supra*.

[0071] One type of adsorbent is a "chromatographic adsorbent," which is a material typically used in chromatography. Chromatographic adsorbents include, for example, ion exchange materials, metal chelators, immobilized metal chelates, hydrophobic interaction adsorbents, hydrophilic interaction adsorbents, dyes, simple biomolecules (e.g., nucleotides, amino acids, simple sugars and fatty acids), mixed mode adsorbents (e.g., hydrophobic attraction/electrostatic repulsion adsorbents).

"Biospecific adsorbent" is another category, for adsorbents that contain a biomolecule, e.g., a nucleotide, a nucleic acid molecule, an amino acid, a polypeptide, a polysaccharide, a lipid, a steroid or a conjugate of these (e.g., a glycoprotein, a lipoprotein, a glycolipid). In certain instances the biospecific adsorbent can be a macromolecular structure such as a multiprotein complex, a biological membrane or a virus. Illustrative biospecific adsorbents are antibodies, receptor proteins, and nucleic acids. A biospecific adsorbent typically has higher specificity for a target analyte than a chromatographic adsorbent.

[0072] Another version of SELDI is Surface-Enhanced Neat Desorption (SEND), which involves the use of probes comprising energy absorbing molecules that are chemically bound to the probe surface ("SEND probe"). The phrase "Energy absorbing molecules" (EAM) denotes molecules that are capable of absorbing energy from a laser desorption ionization source and, thereafter, contributing to desorption and ionization of analyte molecules in contact therewith. The EAM category includes molecules used in MALDI, frequently referred to as "matrix," and is exemplified by cinnamic acid derivatives, sinapinic acid (SPA), cyano-hydroxy-cinnamic acid (CHCA) and dihydroxybenzoic acid, ferulic acid, and hydroxyacetophenone derivatives. The category also includes EAMs used in SELDI, as enumerated, for example, by U.S. 5,719,060 and U.S. 6,073,519 (Kisagawa), filed January 25, 2002.

[0073] Another version of SELDI, called Surface-Enhanced Photolabile Attachment and Release (SEPAR), involves the use of probes having moieties attached to the surface that can covalently bind an analyte, and then release the analyte through breaking a photolabile bond in the moiety after exposure to light, e.g., to laser light.

For instance, see U.S. 5,719,060. SEPAR and other forms of SELDI are readily adapted to detecting a biomarker or biomarker profile, pursuant to the present invention.

[0074] The detection of the biomarkers according to the invention can be enhanced by using certain selectivity conditions, e.g., adsorbents or washing solutions. The phrase "wash solution" refers to an agent, typically a solution, which is used to affect or modify adsorption of an analyte to an adsorbent surface and/or to remove unbound materials from the surface. The elution characteristics of a wash solution can depend, for example, on pH, ionic strength, hydrophobicity, degree of chaotropism, detergent strength, and temperature.

[0075] Pursuant to one aspect of the present invention, a sample is analyzed by means of a "biochip," a term that denotes a solid substrate, having a generally planar surface, to which a capture reagent (adsorbent) is attached. Frequently, the surface of a biochip comprises a plurality of addressable locations, each of which has the capture reagent bound there. A biochip can be adapted to engage a probe interface and, hence, function as a probe in gas phase ion spectrometry preferably mass spectrometry. Alternatively, a biochip of the invention can be mounted onto another substrate to form a probe that can be inserted into the spectrometer.

[0076] A variety of biochips is available for the capture of biomarkers, in accordance with the present invention, from commercial sources such as CIPHERGEN Biosystems (Fremont, CA), Perkin Elmer (Packard BioScience Company (Meriden CT), Zymyx (Hayward, CA), and Phyllos (Lexington, MA). Exemplary of these biochips are those described in U.S. patents No. 6,225,047, *supra*, and No. 6,329,209 (Wagner et al.), and in PCT publications WO 99/51773 (Kulmelis and Wagner) and WO 00/56934 (Englert et al.).

[0077] More specifically, biochips produced by CIPHERGEN Biosystems have surfaces, presented on an aluminum substrate in strip form, to which are attached, at addressable locations, chromatographic or biospecific adsorbents. The surface of the strip is coated with silicon dioxide.

[0078] Illustrative of CIPHERGEN ProteinChip® arrays are biochips H4, SAX-2, WCX-2, and IMAC-3, which include a functionalized, cross-linked polymer in the

form of a hydrogel, physically attached to the surface of the biochip or covalently attached through a silane to the surface of the biochip. The H4 biochip has isopropyl functionalities for hydrophobic binding. The SAX-2 biochip has quaternary ammonium functionalities for anion exchange. The WCX-2 biochip has carboxylate functionalities for cation exchange. The IMAC-3 biochip has nitroacetic acid functionalities that adsorb transition metal ions, such as Cu⁺⁺ and Ni⁺⁺, by chelation. These immobilized metal ions, in turn, allow for adsorption of biomarkers by coordinate covalent bonding. Thus, Ciphergen's IMAC ProteinChip® arrays are sold with reactive moieties that become adsorbent upon the addition by the user of a metal solution.

[0079] In keeping with the above-described principles, a substrate with an adsorbent is contacted with the sample, containing serum, for a period of time sufficient to allow biomarker that may be present to bind to the adsorbent. In one embodiment of the invention, more than one type of substrate with adsorbent thereon is contacted with the biological sample. For example, a sample may be applied to both a WCX and an IMAC chip. This technique can allow for even more definitive assessment of cancer status. After the incubation period, the substrate is washed to remove unbound material. Any suitable washing solutions can be used; preferably, aqueous solutions are employed.

[0080] An energy absorbing molecule then is applied to the substrate with the bound biomarkers. As noted, an energy absorbing molecule is a molecule that absorbs energy from an energy source such as a laser, thereby assisting in desorption of biomarkers from the substrate. Exemplary energy absorbing molecules include, as noted above, cinnamic acid derivatives, sinapinic acid and dihydroxybenzoic acid. Preferably sinapinic acid is used.

[0081] The biomarkers bound to the substrates are detected in a gas phase ion spectrometer such as a time-of-flight mass spectrometer. The biomarkers are ionized by an ionization source such as a laser, the generated ions are collected by an ion optic assembly, and then a mass analyzer disperses and analyzes the passing ions. The detector then translates information of the detected ions into mass-to-charge

ratios. Detection of a biomarker typically will involve detection of signal intensity. Thus, both the quantity and mass of the biomarker can be determined.

[0082] Data generated by desorption and detection of biomarkers can be analyzed with the use of a programmable digital computer. The computer program analyzes the data to indicate the number of markers detected, and optionally the strength of the signal and the determined molecular mass for each biomarker detected. Data analysis can include steps of determining signal strength of a biomarker and removing data deviating from a predetermined statistical distribution. For example, the observed peaks can be normalized, by calculating the height of each peak relative to some reference. The reference can be background noise generated by the instrument and chemicals such as the energy absorbing molecule which is set as zero in the scale. [0083] The computer can transform the resulting data into various formats for display. The standard spectrum can be displayed, but in one useful format only the peak height and mass information are retained from the spectrum view, yielding a cleaner image and enabling biomarkers with nearly identical molecular weights to be more easily seen. In another useful format, two or more spectra are compared, conveniently highlighting unique biomarkers and biomarkers that are up- or down-regulated between samples. Using any of these formats, one can readily determine whether a particular biomarker is present in a sample.

[0084] Software used to analyze the data can include code that applies an algorithm to the analysis of the signal to determine whether the signal represents a peak in a signal that corresponds to a biomarker according to the present invention. The software also can subject the data regarding observed biomarker peaks to classification tree or ANN analysis, to determine whether a biomarker peak or combination of biomarker peaks is present that indicates lung cancer status. Analysis of the data may be "keyed" to a variety of parameters that are obtained either directly or indirectly from the mass spectrometric analysis of the sample. These parameters include, but are not limited to, the presence or absence of one or more peaks, the height of one or more peaks, the log of the height of one or more peaks, and other arithmetic manipulations of peak height data.

[0085] In another aspect, the present invention provides kits for aiding in the diagnosis of lung cancer status, which kits are used to detect biomarkers according to the invention. The kits screen for the presence of biomarkers and combinations of biomarkers that are differentially present in samples from normal subjects and subjects with lung cancer.

[0086] In one embodiment, the kit comprises a substrate having an adsorbent thereon, wherein the adsorbent is suitable for binding a biomarker according to the invention, and a washing solution or instructions for making a washing solution, in which the combination of the adsorbent and the washing solution allows detection of the biomarker using gas phase ion spectrometry, e.g., mass spectrometry. The kit may include more than one type of adsorbent, each present on a different substrate.

[0087] In another embodiment, a kit of the invention may include a first substrate, comprising an adsorbent thereon, and a second substrate onto which the first substrate is positioned to form a probe, which can be inserted into a gas phase ion spectrometer, e.g., a mass spectrometer. In another embodiment, an inventive kit may comprise a single substrate that can be inserted into the spectrometer.

[0088] In a further embodiment, such a kit can comprise instructions for suitable operational parameters in the form of a label or separate insert. For example, the instructions may inform a consumer how to collect the sample or how to wash the probe. In yet another embodiment the kit can comprise one or more containers with biomarker samples, to be used as standard(s) for calibration.

[0089] In a preferred embodiment, the detection of biomarkers for diagnosis of lung cancer in a subject entails contacting a sample from a subject or patient, preferably a serum sample, with a substrate having an adsorbent thereon under conditions that allow binding between the biomarker and the adsorbent, and then detecting the biomarker bound to the adsorbent by gas phase ion spectrometry, preferably by Surface Enhanced Laser Desorption/Ionization (SELDI) mass spectrometry. The biomarkers are ionized by an ionization source such as a laser. The generated ions are collected by an ion optic assembly and accelerated toward an ion detector. Ions that strike the detector generate an electric potential that is digitized by a high speed time-array recording device that digitally captures the analog signal. CIPHERGEN's

ProteinChip® system employs an analog-to-digital converter (ADC) to accomplish this. The ADC integrates detector output at regularly spaced time intervals into time-dependent bins. The time intervals typically are one to four nanoseconds long. Furthermore, the time-of-flight spectrum ultimately analyzed typically does not represent the signal from a single pulse of ionizing energy against a sample, but rather the sum of signals from a number of pulses. This reduces noise and increases dynamic range. This time-of-flight data is then subject to data processing. In CIPHERGEN's ProteinChip® software, data processing typically includes TOF-to-MZ transformation, baseline subtraction, high frequency noise filtering. Thus, both the quantity and mass of the biomarker can be determined.

[0090] The detection of the biomarkers can be enhanced by using certain selectivity conditions, e.g., adsorbents or washing solutions. In one embodiment, the same or similar selectivity conditions that were used to discover the biomarkers are used in the method of detecting the biomarker in the sample. For example, immobilized metal affinity capture chips such as the Cu(II) IMAC3 and weak cationic exchange chips such as the WCX2 chips are preferred as the adsorbents for biomarker detection. However, other adsorbents can be used, as long as they have the binding characteristics suitable for binding the biomarkers.

[0091] More particularly, armed with the information regarding the biomarkers identified herein, various methods can be used to recognize patterns of doublets, triplets, and higher combinations of biomarkers according to the invention. These methods take raw data regarding which peaks are present and their intensity and provide a differential diagnosis of lung cancer versus normal for a sample.

[0092] Thus, the process can be divided into the learning phase and the classification phase. In the learning phase, a learning algorithm is applied to a data set that includes members of the different classes that are meant to be classified, for example, data from a plurality of samples diagnosed as cancer and data from a plurality of samples assigned a negative diagnosis. The methods used to analyze the data include, but are not limited to, artificial neural network, support vector machines, genetic algorithm and self-organizing maps and classification and regression tree analysis. These methods are described, for example, in WO 01/31579, May 3, 2001

(Barnhill *et al.*; WO 02/06829, January 24, 2002 (Hitt *et al.*) and WO 02/42733, May 30, 2002 (Paulse *et al.*). The learning algorithm produces a classifying algorithm. The classifier is keyed to elements of the data, such as particular markers and particular intensities of markers, usually in combination, that can classify an unknown sample into one of the two classes. The classifier is ultimately used for diagnostic testing.

[0093] Software, both freeware and proprietary software, is readily available to analyze such patterns in data, and to devise additional patterns with any predetermined criteria for success. Those biomarkers which by themselves are predictive of a differential diagnosis of lung cancer versus normal do not require pattern recognition software to analyze the data.

[0094] The following examples are offered by way of illustration, and are not limiting.

Example I. Fractionation of serum

Buffers:

1. U9 (9M urea, 2% CHAPS, 50mM Tris-HCl pH9)
2. U1 (1M urea, 0.22% CHAPS, 50mM Tris-HCl pH9)
3. wash buffer 1: 50mM Tris-HCl with 0.1% n-octyl β -D-Glucopyranoside (OGP) pH9
4. wash buffer 2: 100mM sodium phosphate with 0.1% OGP pH7
5. wash buffer 3: 100mM sodium acetate with 0.1% OGP pH5
6. wash buffer 4: 100mM sodium acetate with 0.1% OGP pH4
7. wash buffer 5: 50mM sodium citrate with 0.1% OGP pH3
8. wash buffer 6: 33.3% isopropanol / 16.7% acetonitrile / 0.1% trifluoroacetic acid in water.

[0095] Thirty microliters of U9 buffer were added to 20 μ L of serum in a tube and were mixed at 4°C for 20 minutes. Ion exchange resin (Q Ceramic HyperD[®] ion exchange resin, Bioserra SA, France) was washed 3 times with 5 bed volumes of 50mM Tris-HCl pH9 and stored in 50% suspension. To each well of a 96-well filter plate (96-well Silent Screen filter plate, Immodyne membrane, 0.45 micron pore,

Nalge Nunc International, USA), 125 μ L of ion exchange resin (50% suspension) was added on a Biomek 2000 Automation Workstation (Beckman Coulter, Fullerton, CA), washed 3 times with 150 μ L U1 buffer, and vacuum dried. Urea-treated serum was transferred to each well of ion exchange resin. The serum tube was rinsed with 50 μ L of U1 buffer, which was also transferred to the corresponding well in filter plate. The filter plate was mixed on a platform shaker at 4°C for 30 minutes. Flow-through fraction was collected in a 96-well plate by vacuum suction (Fraction 1). Then, 100 μ L of wash buffer 1 was added to each well of filter plate and mixed for 10 minutes at room temperature. Eluant was collected into the same 96-well plate (Fraction 1). Resins in the filter plate were subsequently washed two times each with 100 μ L wash buffers 2, 3, 4, 5 and 6. Each eluant (total volume of 200 μ L) was collected in a 96-well plate (Fractions 2,3,4,5 and 6).

Example 2. SELDI analysis of fractionated serum

[0096] ProteinChip[®] Arrays were set up in 96-well bioprocessors. Buffer delivery and sample incubation were performed on a Biomek 2000 Automation Workstation. Each serum fraction was analyzed on IMAC3 (loaded with copper) and WCX2 ProteinChip[®] Arrays in duplicates. IMAC3 copper and WCX2 arrays (Ciphergen Biosystems Inc, Fremont, CA) were equilibrated two times with 150 μ L of binding buffer (100mM sodium phosphate + 0.5M NaCl pH7 for IMAC3, 100mM sodium acetate pH4 for WCX2). Each serum fraction was diluted in the corresponding binding buffer (1/5 dilution for IMAC3 and 1/10 dilution for WCX2) and 100 μ L was applied to each ProteinChip[®] array. Incubation was performed on a platform shaker at room temperature for 30 minutes. Each array was washed three times with 150 μ L of corresponding binding buffer and rinsed two times with water. ProteinChip[®] arrays were air-dried. Sinapinic acid matrix (prepared in 50% acetonitrile, 0.5% trifluoroacetic acid) was applied to each array. ProteinChip[®] arrays were read on a ProteinChip[®] PBSII Reader (Ciphergen Biosystems Inc.) A total of 253 laser shots were averaged for each array.

[0097] All publications and patent documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if

each individual publication or patent document were so individually denoted. By their citation of various references in this document Applicants do not admit that any particular reference is "prior art" to their invention.

What we claim is:

1. A method for qualifying lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a diagnostic level of a protein selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310,

wherein the biomarker is differentially present in samples of a subject with lung cancer and a normal subject that is free of lung cancer.

2. The method according to claim 1, wherein the protein is selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, and IM-155,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, and WM-70.

3. The method according to claim 1, wherein the protein is selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, and IM-454,

or from a second group consisting

(ii) WM-61, WM-447, WM-446, WM-133, and WM-119.

4. The method according to claim 1, which uses a single biomarker

selected from the group consisting of the WM-446 and WM-447.

5. A method for qualifying lung carcinoma risk in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310.

6. The method according to claim 5, wherein the pattern-recognition analysis is keyed to a pair of peaks selected either from a first group consisting of

(i) IM-266 and IM-474, IM-266 and IM-38, IM-266 and IM-454, IM-266 and IM-522, IM-266 and IM-544, IM-266 and IM-471, IM-474 and IM-151, IM-474 and IM-156, IM-474 and IM-544, IM-474 and IM-38, IM-522 and IM-507, IM-522 and IM-156, and IM-522 and IM-440;

or from a second group consisting of

(ii) WM-447 and WM-59, WM-447 and WM-19, WM-447 and WM-118, WM-447 and WM-473, WM-19 and WM-59, WM-19 and WM-473, WM-19 and WM-369, WM-61 and WM-154, WM-61 and WM-369, WM-118 and WM-59 and WM-282 and WM-127.

7. The method according to claim 5, wherein the pattern-recognition analysis is keyed to a pair of peaks selected from either a first group consisting of

(i) IM-266 and IM-474, IM-266 and IM-544, and IM-156 and IM-522;

or from a second group consisting of

(ii) WM-447 and WM-59, WM-447 and WM-19, and WM-19 and WM-59.

8. The method according to claim 5, wherein the pattern-recognition analysis is keyed to a triplet of peaks selected from

(i) IM-266, IM-454 and IM-474; and IM-266, IM-474 and IM-544;

or wherein the analysis is keyed to

(ii) WM-447, WM-19 and WM-473.

9. A kit for detecting and diagnosing lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

10. A kit according to claim 9, further comprising a washing solution or instructions for making a washing solution.

11. A kit according to claim 9, wherein the substrate is a SELDI probe that comprises either (i) functionalities that adsorb transition metal ions by chelation or (ii) functionalities that allow for cation exchange.

12. A method for qualifying lung adenocarcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, WM-120, WM-134, WM-276, WM-428, WM-277, WM-20, WM-119, WM-340, WM-48, WM-389, WM-450, WM-47, WM-343, WM-17, WM-583, WM-70, WM-706, WM-346, WM-466, WM-646, WM-384, WM-336, WM-294, WM-339, WM-473, WM-369, WM-38, WM-283, WM-685, WM-66, WM-55, WM-650, WM-307, WM-278, WM-342, and WM-429.

13. The method according to claim 12, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, and WM-120.

14. The method according to claim 12, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290.

15. A method for qualifying status of lung adenocarcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, WM-120, WM-134, WM-276, WM-428, WM-277, WM-20, WM-119, WM-340, WM-48, WM-

389, WM-450, WM-47, WM-343, WM-17, WM-583, WM-70, WM-706, WM-346, WM-466, WM-646, WM-384, WM-336, WM-294, WM-339, WM-473, WM-369, WM-38, WM-283, WM-685, WM-66, WM-55, WM-650, WM-307, WM-278, WM-342, and WM-429.

16. The method according to claim 15, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, and WM-120.

17. The method according to claim 15, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290.

18. A kit for detecting and diagnosing lung adenocarcinoma, comprising (A) an adsorbent attached to a substrate that retains one or more of biomarkers selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, WM-120, WM-134, WM-276, WM-428, WM-277, WM-20, WM-119, WM-340, WM-48, WM-389, WM-450, WM-47, WM-343, WM-17, WM-583, WM-70, WM-706, WM-346, WM-466, WM-646, WM-384, WM-336, WM-294, WM-339, WM-473, WM-369, WM-38, WM-283, WM-685, WM-66, WM-55, WM-650, WM-307, WM-278, WM-342, and WM-429, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

19. A kit according to claim 18, further comprising a washing solution or instructions for making a washing solution.

20. A kit according to claim 18, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

21. A method for qualifying squamous cell lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, WM-70, WM-341, WM-429, WM-347, WM-17, WM-47, WM-431, WM-62, WM-473, WM-384, WM-438, WM-652, WM-282, WM-389, WM-290,

WM-278, WM-456, WM-673, WM-340, WM-55, WM-455, WM-645, WM-138, WM-420, WM-450, WM-369, WM-279, WM-342, WM-471, WM-674, WM-120, WM-20, WM-287, WM-83, WM-154, and WM-128.

22. The method according to claim 21, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, and WM-70.

23. The method according to claim 21, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, and WM-133.

24. A method for qualifying status of squamous cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, WM-70, WM-341, WM-429, WM-347, WM-17, WM-47, WM-431, WM-62, WM-473, WM-384, WM-438, WM-652, WM-282, WM-389, WM-290, WM-278, WM-456, WM-673, WM-340, WM-55, WM-455, WM-645, WM-138, WM-420, WM-450, WM-369, WM-279, WM-342, WM-471, WM-674, WM-120, WM-20, WM-287, WM-83, WM-154, and WM-128.

25. The method according to claim 24, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, and WM-70.

26. The method according to claim 24, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, and WM-133.

27. A kit for detecting and diagnosing squamous cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, WM-70, WM-341, WM-429, WM-347, WM-17, WM-47, WM-431, WM-62, WM-473, WM-384, WM-438, WM-652, WM-282, WM-389, WM-290, WM-278, WM-456, WM-673, WM-340, WM-55, WM-455, WM-645, WM-138, WM-420, WM-450, WM-369, WM-279, WM-342, WM-471, WM-674, WM-120, WM-20, WM-287, WM-83, WM-154, and WM-128, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

28. A kit according to claim 27, further comprising a washing solution or instructions for making a washing solution.

29. A kit according to claim 27, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

30. A method for qualifying small cell lung carcinoma status in a subject, comprising analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, WM-685, WM-473, WM-343, WM-466, WM-341, WM-340, WM-363, WM-339, WM-457, WM-86, WM-506, WM-72, WM-287, WM-82, WM-528, WM-85, WM-73, WM-138, WM-384, WM-83, WM-450, WM-310, WM-277, WM-79, WM-207, WM-278, WM-290, WM-366, WM-472, WM-420, WM-147, WM-55, WM-669, WM-357, WM-429, and WM-279.

31. The method according to claim 30, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, and WM-685.

32. The method according to claim 30, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, and WM-61.

33. A method for qualifying status of small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-

recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, WM-685, WM-473, WM-343, WM-466, WM-341, WM-340, WM-363, WM-339, WM-457, WM-86, WM-306, WM-72, WM-287, WM-82, WM-528, WM-85, WM-73, WM-138, WM-384, WM-83, WM-450, WM-310, WM-277, WM-79, WM-207, WM-278, WM-290, WM-366, WM-472, WM-420, WM-147, WM-55, WM-669, WM-357, WM-429, and WM-279.

34. The method according to claim 33, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, and WM-685.

35. The method according to claim 33, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, and WM-61.

36. A kit for detecting and diagnosing small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, WM-685, WM-473, WM-343, WM-466, WM-341, WM-340, WM-363, WM-339, WM-457, WM-86, WM-506, WM-72, WM-287, WM-82, WM-528, WM-85, WM-73, WM-138, WM-384, WM-83, WM-450, WM-310, WM-277, WM-79, WM-207, WM-278, WM-290, WM-366, WM-472, WM-420, WM-147, WM-55, WM-669, WM-357, WM-429, and WM-279, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

37. A kit according to claim 36, further comprising a washing solution or instructions for making a washing solution.

38. A kit according to claim 36, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

39. A method for qualifying non-small cell lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, WM-456, WM-450, WM-283, WM-207, WM-436, WM-384, WM-61, WM-167, WM-382, WM-285, WM-650, WM-203, WM-119, WM-282, WM-686, WM-383, WM-429, WM-11, WM-208, WM-451, WM-473, WM-220, WM-685, WM-338, WM-71, WM-266, WM-70, WM-545, WM-675, WM-446, WM-120, WM-267, WM-466, WM-347, WM-153, and WM-38.

40. The method according to claim 39, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, and WM-456.

41. The method according to claim 39, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, and WM-340.

42. A method for qualifying status of non-small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, WM-456, WM-450, WM-283, WM-207, WM-436, WM-384, WM-61, WM-167, WM-382, WM-285, WM-650, WM-203, WM-119, WM-282, WM-686, WM-383, WM-429, WM-11, WM-208, WM-451, WM-473, WM-220, WM-685, WM-338, WM-71, WM-266, WM-70, WM-545, WM-675, WM-446, WM-120, WM-267, WM-466, WM-347, WM-153, and WM-38.

43. The method according to claim 42, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, and WM-456.

44. The method according to claim 42, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, and WM-340.

45. A kit for detecting and diagnosing non-small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, WM-456, WM-450, WM-283, WM-207, WM-436, WM-384, WM-61, WM-167, WM-382, WM-285, WM-650, WM-203, WM-119, WM-282, WM-686, WM-383, WM-429, WM-11, WM-208, WM-451, WM-473, WM-220, WM-685, WM-338, WM-71, WM-266, WM-70, WM-545, WM-675, WM-446, WM-120, WM-267, WM-466, WM-347, WM-153, and WM-338, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

46. A kit according to claim 45, further comprising a washing solution or instructions for making a washing solution.

47. A kit according to claim 45, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

48. A method for qualifying large cell lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488.

49. The method according to claim 48, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, and WM-447.

50. The method according to claim 48, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, and WM-647.

51. A method for qualifying status of large cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488.

52. The method according to claim 51, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, and WM-447.

53. The method according to claim 51, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, and WM-647.

54. A kit for detecting and diagnosing large cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-

545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

55. A kit according to claim 50, further comprising a washing solution or instructions for making a washing solution.

56. A kit according to claim 50, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

57. A method for distinguishing lung adenocarcinoma from squamous lung carcinoma in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, WM-211, WM-362, WM-133, WM-414, WM-277, WM-141, WM-64, WM-135, WM-447, WM-383, WM-338, WM-63, WM-142, WM-446, WM-186, WM-111, WM-445, WM-455, WM-276, WM-444, WM-181, WM-35, WM-285, WM-456, WM-39, WM-82, WM-17, WM-203, WM-83, WM-412, WM-96, WM-74, WM-457, WM-431, WM-340, and WM-49.

58. The method according to claim 57, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, and WM-211.

59. The method according to claim 57, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, and WM-347.

60. A method for distinguishing lung adenocarcinoma from squamous lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from the group

consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, WM-211, WM-362, WM-133, WM-414, WM-277, WM-141, WM-64, WM-135, WM-447, WM-383, WM-338, WM-63, WM-142, WM-446, WM-186, WM-111, WM-445, WM-455, WM-276, WM-444, WM-181, WM-35, WM-285, WM-456, WM-39, WM-82, WM-17, WM-203, WM-83, WM-412, WM-96, WM-74, WM-457, WM-431, WM-340, and WM-49.

61. The method according to claim 60, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, and WM-211.

62. The method according to claim 60, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, and WM-347.

63. A kit for distinguishing lung adenocarcinoma from squamous lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

64. A kit according to claim 63, further comprising a washing solution or instructions for making a washing solution.

65. A kit according to claim 63, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

66. A method for distinguishing lung adenocarcinoma from small cell lung carcinoma in a subject, comprised of analyzing a biological sample from said subject

for a level of a protein selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, WM-455, WM-313, WM-456, WM-86, WM-70, WM-246, WM-360, WM-190, WM-418, WM-83, WM-257, WM-138, WM-47, WM-252, WM-282, WM-60, WM-68, WM-325, WM-402, WM-411, WM-405, WM-75, WM-417, WM-387, WM-56, WM-410, WM-420, WM-164, WM-67, WM-66, WM-391, WM-340, WM-428, WM-198, WM-312, and WM-152.

67. The method according to claim 66, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, and WM-455.

68. The method according to claim 66, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, and WM-79.

69. A method for distinguishing lung adenocarcinoma from small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, WM-455, WM-313, WM-456, WM-86, WM-70, WM-246, WM-360, WM-190, WM-418, WM-83, WM-257, WM-138, WM-47, WM-252, WM-282, WM-60, WM-68, WM-325, WM-402, WM-411, WM-405, WM-75, WM-417, WM-387, WM-26, WM-410, WM-420, WM-164, WM-67, WM-66, WM-391, WM-340, WM-428, WM-198, WM-312, and WM-152.

70. The method according to claim 69, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, and WM-455.

71. The method according to claim 69, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79.

72. A kit for distinguishing lung adenocarcinoma from small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447, WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157, WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301, WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288, WM-384, and WM-37, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

73. A kit according to claim 72, further comprising a washing solution or instructions for making a washing solution.

74. A kit according to claim 72, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

75. A method for distinguishing squamous cell lung carcinoma from small cell lung carcinoma in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447, WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157, WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301, WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288, WM-384, and WM-37.

76. The method according to claim 75, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, and WM-447.

77. The method according to claim 75, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, and WM-363.

78. A method for distinguishing squamous cell lung carcinoma from small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-

recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53,

WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447,

WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-

580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157,

WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301,

WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288,

WM-384, and WM-37.

79. The method according to claim 78, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, and WM-447.

80. The method according to claim 78, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, and WM-363.

81. A kit for distinguishing squamous cell lung carcinoma from small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the

biomarkers WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447,

WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-

580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157,

WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301,

WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288,

WM-384, and WM-37, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

82. A kit according to claim 81, further comprising a washing solution or instructions for making a washing solution.

83. A kit according to claim 81, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

84. Software for qualifying lung carcinoma status in a subject, comprising an algorithm for analyzing data extracted from a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, wherein said data relates to one or more biomarkers selected from either a first group consisting of:

(i) WM-522, WM-273, WM-520, WM-519, WM-454, WM-507, WM-521, WM-148, WM-266, WM-537, WM-471, WM-510, WM-544, WM-474, WM-155, WM-157, WM-176, WM-445, WM-177, WM-440, WM-468, WM-438, WM-547, WM-359, WM-436, WM-106, WM-455, WM-444, WM-158, WM-265, WM-50, WM-159, WM-156, WM-439, WM-157, WM-508, WM-514, WM-478, WM-473, WM-360, WM-435, WM-150, WM-151, WM-110, WM-51, WM-163, WM-437, WM-546, WM-153, and WM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310.

85. Software according to claim 84, wherein said algorithm carries out a pattern-recognition analysis that is keyed to data relating to at least one of the biomarkers.

86. Software according to claim 85, wherein said algorithm comprises classification tree analysis that is keyed to data relating to at least one of the biomarkers.

87. Software according to claim 85, wherein said algorithm comprises artificial neural network analysis that is keyed to data relating to at least one of the biomarkers.

88. A method for qualifying lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a diagnostic level of a biomarker that is serum amyloid A protein or a fragment thereof.

89. A method according to claim 88, wherein said serum biomarker has an apparent molecular weight of about 2803, 3168, 3277, 3552, 3897, 4300, 4490, 4655, 5927, 6874, 7776, 7941, 8152, 8952, 9233, 10300, 10866, or 10851 Daltons.

90. A method according to claim 89, wherein said serum biomarker has an apparent molecular weight of about 3168, 3277, 3552, 3897, 4300, 4490, 4655, 7776, 7941, 8152, 8952, or 10851 Daltons.

91. A method according to claim 88, wherein said serum biomarker has an apparent molecular weight of about 11.5 to 11.7 kD.

92. A method according to claim 88, for qualifying risk of lung adenocarcinoma.

93. A method according to claim 88, for qualifying risk of squamous cell lung carcinoma.

94. A method according to claim 88, for qualifying risk of small cell lung carcinoma.

95. A method according to claim 88, for qualifying risk of non-small cell lung carcinoma.

96. A method according to claim 88, for qualifying risk of large cell lung carcinoma.

97. A kit for detecting and diagnosing lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers that are serum amyloid A protein or a fragment thereof and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

98. A kit according to claim 97, wherein said serum biomarker has an apparent molecular weight of about 2803, 3168, 3277, 3552, 3897, 4300, 4490, 4655, 5927, 6874, 7776, 7941, 8152, 8952, 9233, 10300, 10866, or 10851 Daltons.

99. A kit according to claim 98, wherein said serum biomarker has an apparent molecular weight of about 3168, 3277, 3552, 3897, 4300, 4490, 4655, 7776, 7941, 8152, 8952, or 10851 Daltons.

100. A kit according to claim 97, wherein said serum biomarker has an apparent molecular weight of about 11.5 to 11.7 kD.

101. A kit according to claim 97, further comprising a washing solution or instructions for making a washing solution.

102. A kit according to claim 97, wherein the substrate is a SELDI probe.

FIGURE 1A

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-1	2011	A	IM-37	3893	A	IM-72	54028	A	IM-109	2882	B
IM-2	2030	A	IM-38	3860	A	IM-73	60170	A	IM-110	2967	B
IM-3	2069	A	IM-39	3972	A	IM-75	74372	A	IM-111	2977	B
IM-4	2128	A	IM-40	3984	A	IM-76	75545	A	IM-112	2994	B
IM-5	2146	A	IM-41	4088	A	IM-77	77543	A	IM-113	3031	B
IM-6	2188	A	IM-42	4178	A	IM-78	79507	A	IM-114	3048	B
IM-7	2232	A	IM-43	4287	A	IM-79	89854	A	IM-115	3148	B
IM-8	2277	A	IM-44	4287	A	IM-80	101831	A	IM-116	3168	B
IM-9	2295	A	IM-45	4309	A	IM-81	104301	A	IM-117	3263	B
IM-10	2318	A	IM-46	4484	A	IM-82	125160	A	IM-118	3308	B
IM-11	2411	A	IM-47	4649	A	IM-83	132976	A	IM-119	3332	B
IM-12	2434	A	IM-48	4798	A	IM-84	149099	A	IM-120	3432	B
IM-13	2467	A	IM-49	5104	A	IM-85	2016	B	IM-121	3450	B
IM-14	2482	A	IM-50	5918	A	IM-86	2029	B	IM-122	3581	B
IM-15	2498	A	IM-51	6122	A	IM-87	2144	B	IM-123	3615	B
IM-16	2565	A	IM-52	6192	A	IM-88	2130	B	IM-124	3714	B
IM-17	2574	A	IM-53	6452	A	IM-89	2188	B	IM-125	3730	B
IM-18	2586	A	IM-54	6660	A	IM-90	2184	B	IM-126	3834	B
IM-19	2605	A	IM-55	7768	A	IM-91	2200	B	IM-127	3899	B
IM-20	2722	A	IM-56	8145	A	IM-92	2284	B	IM-128	3969	B
IM-21	2746	A	IM-57	8954	A	IM-93	2289	B	IM-129	3986	B
IM-22	2788	A	IM-58	9312	A	IM-94	2314	B	IM-130	3997	B
IM-23	2855	A	IM-59	9449	A	IM-95	2414	B	IM-131	4013	B
IM-24	2871	A	IM-60	10272	A	IM-96	2428	B	IM-132	4181	B
IM-25	2984	A	IM-61	11683	A	IM-97	2451	B	IM-133	4297	B
IM-26	3030	A	IM-62	13376	A	IM-98	2468	B	IM-134	4311	B
IM-27	3144	A	IM-63	14698	A	IM-99	2483	B	IM-135	4465	B
IM-28	3243	A	IM-64	15190	A	IM-100	2565	B	IM-136	4484	B
IM-29	3273	A	IM-65	66758	A	IM-101	2583	B	IM-137	4579	B
IM-30	3290	A	IM-66	15951	A	IM-102	2597	B	IM-138	4608	B
IM-31	3369	A	IM-67	15172	A	IM-103	2697	B	IM-139	4669	B
IM-32	3445	A	IM-68	15925	A	IM-104	2715	B	IM-140	4747	B
IM-33	3483	A	IM-69	23438	A	IM-105	2740	B	IM-141	4882	B
IM-34	3676	A	IM-70	39784	A	IM-106	2752	B	IM-142	4891	B
IM-35	3779	A	IM-71	44188	A	IM-107	2767	B	IM-143	5033	B
IM-36	3793	A		46890	A	IM-108	2885	B	IM-144	5077	B

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FIGURE 1B

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-145	5099	B	IM-181	16018	B	IM-217	2130	C	IM-253	3733	C
IM-146	5143	B	IM-182	17346	B	IM-218	2145	C	IM-254	3833	C
IM-147	5158	B	IM-183	18311	B	IM-219	2167	C	IM-255	3900	C
IM-148	5272	B	IM-184	22588	B	IM-220	2182	C	IM-256	4010	C
IM-149	5306	B	IM-185	23422	B	IM-221	2199	C	IM-257	4145	C
IM-150	5349	B	IM-186	27969	B	IM-222	2211	C	IM-258	4187	C
IM-151	5384	B	IM-187	33283	B	IM-223	2230	C	IM-259	4289	C
IM-152	5421	B	IM-188	39808	B	IM-224	2250	C	IM-260	4488	C
IM-153	5554	B	IM-189	43110	B	IM-225	2280	C	IM-261	4582	C
IM-154	5711	B	IM-190	44454	B	IM-226	2297	C	IM-262	4813	C
IM-155	5878	B	IM-191	47215	B	IM-227	2317	C	IM-263	4876	C
IM-156	5918	B	IM-192	53784	B	IM-228	2412	C	IM-264	5032	C
IM-157	5931	B	IM-193	55952	B	IM-229	2428	C	IM-265	5347	C
IM-158	5988	B	IM-194	60573	B	IM-230	2468	C	IM-266	5365	C
IM-159	6137	B	IM-195	66346	B	IM-231	2481	C	IM-267	5632	C
IM-160	6200	B	IM-196	73387	B	IM-232	2498	C	IM-268	7767	C
IM-161	6443	B	IM-197	79203	B	IM-233	2587	C	IM-269	7973	C
IM-162	6644	B	IM-198	89302	B	IM-234	2585	C	IM-270	8143	C
IM-163	6958	B	IM-199	84226	B	IM-235	2599	C	IM-271	9187	C
IM-164	7481	B	IM-200	99338	B	IM-236	2698	C	IM-272	9293	C
IM-165	7568	B	IM-201	102098	B	IM-237	2715	C	IM-273	11705	C
IM-166	7765	B	IM-202	107189	B	IM-238	2745	C	IM-274	14041	C
IM-167	7955	B	IM-203	116936	B	IM-239	2786	C	IM-275	15114	C
IM-168	8144	B	IM-204	119487	B	IM-240	2887	C	IM-276	15939	C
IM-169	8698	B	IM-205	122103	B	IM-241	2885	C	IM-277	22321	C
IM-170	8821	B	IM-206	125431	B	IM-242	2998	C	IM-278	28001	C
IM-171	8944	B	IM-207	132052	B	IM-243	3052	C	IM-279	33298	C
IM-172	9138	B	IM-208	138518	B	IM-244	3096	C	IM-280	39770	C
IM-173	9298	B	IM-209	145147	B	IM-245	3151	C	IM-281	44460	C
IM-174	9390	B	IM-210	157502	B	IM-246	3167	C	IM-282	47307	C
IM-175	9516	B	IM-211	168578	B	IM-247	3286	C	IM-283	50625	C
IM-176	11711	B	IM-212	173391	B	IM-248	3303	C	IM-284	55898	C
IM-177	11914	B	IM-213	2011	C	IM-249	3335	C	IM-285	60882	C
IM-178	14033	B	IM-214	2030	C	IM-250	3448	C	IM-286	66294	C
IM-179	15110	B	IM-215	2050	C	IM-251	3619	C	IM-287	78892	C
IM-180	15838	B	IM-216	2096	C	IM-252	3709	C	IM-288	83848	C

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MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-289	89081	C	IM-325	2565	D	IM-381	13857	D	IM-397	2082	E
IM-290	94147	C	IM-326	2582	D	IM-382	14058	D	IM-398	2128	E
IM-291	89324	C	IM-327	2587	D	IM-383	15108	D	IM-399	2148	E
IM-292	107183	C	IM-328	2716	D	IM-384	15844	D	IM-400	2170	E
IM-293	110350	C	IM-329	2747	D	IM-365	22243	D	IM-401	2187	E
IM-294	113339	C	IM-330	2787	D	IM-366	25465	D	IM-402	2206	E
IM-295	116291	C	IM-331	2868	D	IM-367	28022	D	IM-403	2232	E
IM-296	122789	C	IM-332	2882	D	IM-368	33272	D	IM-404	2250	E
IM-297	131908	C	IM-333	2894	D	IM-369	40149	D	IM-405	2279	E
IM-298	146248	C	IM-334	3032	D	IM-370	43113	D	IM-406	2296	E
IM-299	159252	C	IM-335	3050	D	IM-371	44219	D	IM-407	2314	E
IM-300	165164	C	IM-336	3148	D	IM-372	47196	D	IM-408	2354	E
IM-301	174928	C	IM-337	3164	D	IM-373	51062	D	IM-409	2394	E
IM-302	186003	C	IM-338	3278	D	IM-374	56082	D	IM-410	2413	E
IM-303	2007	D	IM-339	3334	D	IM-375	58239	D	IM-411	2438	E
IM-304	2016	D	IM-340	3385	D	IM-376	60285	D	IM-412	2457	E
IM-305	2030	D	IM-341	3432	D	IM-377	66148	D	IM-413	2466	E
IM-306	2052	D	IM-342	3451	D	IM-378	73668	D	IM-414	2489	E
IM-307	2089	D	IM-343	3617	D	IM-379	77433	D	IM-415	2568	E
IM-308	2130	D	IM-344	3701	D	IM-380	79985	D	IM-416	2583	E
IM-309	2144	D	IM-345	3725	D	IM-381	80844	D	IM-417	2612	E
IM-310	2154	D	IM-346	3833	D	IM-382	88962	D	IM-418	2662	E
IM-311	2166	D	IM-347	3889	D	IM-383	94399	D	IM-419	2723	E
IM-312	2184	D	IM-348	4008	D	IM-384	99419	D	IM-420	2738	E
IM-313	2204	D	IM-349	4157	D	IM-385	108395	D	IM-421	2750	E
IM-314	2231	D	IM-350	4297	D	IM-386	116433	D	IM-422	2849	E
IM-315	2252	D	IM-351	4580	D	IM-387	123337	D	IM-423	2867	E
IM-316	2275	D	IM-352	4805	D	IM-388	131977	D	IM-424	3036	E
IM-317	2296	D	IM-353	6946	D	IM-389	145658	D	IM-425	3147	E
IM-318	2316	D	IM-354	7053	D	IM-390	152603	D	IM-426	3281	E
IM-319	2412	D	IM-355	7767	D	IM-391	158524	D	IM-427	3319	E
IM-320	2435	D	IM-356	7964	D	IM-392	186072	D	IM-428	3446	E
IM-321	2468	D	IM-357	8139	D	IM-393	2010	E	IM-429	3693	E
IM-322	2480	D	IM-358	8292	D	IM-394	2029	E	IM-430	3731	E
IM-323	2499	D	IM-359	11671	D	IM-395	2050	E	IM-431	3818	E
IM-324	2518	D	IM-360	13727	D	IM-396	2068	E	IM-432	3885	E

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FIGURE 10

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-433	4138	E	IM-469	86211	E	IM-505	4174	F	IM-541	95033	F
IM-434	4169	E	IM-470	89407	E	IM-506	4362	F	IM-542	100310	F
IM-435	4257	E	IM-471	100270	E	IM-507	4473	F	IM-543	118889	F
IM-436	4277	E	IM-472	109638	E	IM-508	4631	F	IM-544	132711	F
IM-437	4355	E	IM-473	117132	E	IM-509	4822	F	IM-545	147276	F
IM-438	4369	E	IM-474	132843	E	IM-510	5882	F	IM-546	160768	F
IM-439	4470	E	IM-475	147160	E	IM-511	6192	F			
IM-440	4488	E	IM-476	152199	E	IM-512	6941	F			
IM-441	4541	E	IM-477	168461	E	IM-513	7626	F			
IM-442	4634	E	IM-478	176835	E	IM-514	7772	F			
IM-443	4841	E	IM-479	2011	F	IM-515	7957	F			
IM-444	5862	E	IM-480	2030	F	IM-516	8150	F			
IM-445	6911	E	IM-481	2128	F	IM-517	8954	F			
IM-446	6849	E	IM-482	2149	F	IM-518	9300	F			
IM-447	6952	E	IM-483	2188	F	IM-519	11545	F			
IM-448	7769	E	IM-484	2207	F	IM-520	11717	F			
IM-449	8148	E	IM-485	2279	F	IM-521	13887	F			
IM-450	8280	E	IM-486	2299	F	IM-522	14073	F			
IM-451	8785	E	IM-487	2319	F	IM-523	15198	F			
IM-452	9301	E	IM-488	2412	F	IM-524	15903	F			
IM-453	10071	E	IM-489	2434	F	IM-525	22460	F			
IM-454	11721	E	IM-490	2487	F	IM-526	23135	F			
IM-455	13910	E	IM-491	2485	F	IM-527	28135	F			
IM-456	15919	E	IM-492	2582	F	IM-528	33577	F			
IM-457	22422	E	IM-493	2605	F	IM-529	39813	F			
IM-458	26233	E	IM-494	2697	F	IM-530	42344	F			
IM-459	33490	E	IM-495	2751	F	IM-531	43274	F			
IM-460	43121	E	IM-496	2885	F	IM-532	44345	F			
IM-461	44558	E	IM-497	3038	F	IM-533	51007	F			
IM-462	46894	E	IM-498	3151	F	IM-534	56318	F			
IM-463	50954	E	IM-499	3372	F	IM-535	60079	F			
IM-464	64478	E	IM-500	3440	F	IM-536	66890	F			
IM-465	60041	E	IM-501	3488	F	IM-537	75122	F			
IM-466	68852	E	IM-502	3717	F	IM-538	78429	F			
IM-467	76580	E	IM-503	3890	F	IM-539	81249	F			
IM-468	79463	E	IM-504	4155	F	IM-540	89384	F			

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FIGURE 2

RANK	MW	MARKER ID	RANK	MW	MARKER ID
1	14073	IM-622	39	117132	IM-473
2	11705	IM-273	40	13727	IM-360
3	11717	IM-520	41	4257	IM-435
4	11545	IM-519	42	5349	IM-150
5	11721	IM-454	43	5384	IM-151
6	4473	IM-507	44	2867	IM-110
7	13887	IM-521	45	6122	IM-51
8	5272	IM-148	46	6958	IM-163
9	5365	IM-266	47	4355	IM-437
10	75122	IM-537	48	180758	IM-548
11	100270	IM-471	49	5554	IM-153
12	5862	IM-510	50	7767	IM-268
13	132711	IM-544			
14	132843	IM-474			
15	5876	IM-155			
16	5832	IM-157			
17	11711	IM-176			
18	5911	IM-445			
19	11914	IM-177			
20	4486	IM-440			
21	78463	IM-468			
22	4369	IM-438			
23	100310	IM-542			
24	11671	IM-359			
25	4277	IM-436			
26	2752	IM-106			
27	13910	IM-455			
28	5862	IM-444			
29	6988	IM-158			
30	5347	IM-265			
31	5918	IM-50			
32	6137	IM-159			
33	5916	IM-156			
34	4470	IM-439			
35	5931	IM-167			
36	4631	IM-508			
37	7772	IM-514			
38	176835	IM-478			

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Figure 3A

Point ID	Fraction	Normal vs Control	Adipose vs Normal	Synovium vs Normal	Small Cell vs Normal	Non-small Cell vs Normal	Large Cell vs Normal	Adipose vs Synovium	Adipose vs Small Cell	Synovium vs Small Cell
WMA-1	A	3070	2011	2019	2011	2020	2011	2020	2010	2010
WMA-2	A	3022	2030	2031		2042	2042	2039		
WMA-3	A	3022	2030	2031	2037	2042	2042	2039	2030	2030
WMA-4	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-5	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-6	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-7	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-8	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-9	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-10	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-11	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-12	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-13	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-14	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-15	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-16	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-17	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-18	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-19	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-20	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-21	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-22	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-23	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-24	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-25	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-26	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-27	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-28	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-29	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-30	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-31	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-32	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-33	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-34	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-35	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-36	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-37	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-38	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-39	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-40	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-41	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-42	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-43	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-44	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-45	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-46	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-47	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-48	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-49	A	2030	2037	2038	2036	2038	2038	2036	2036	2036
WMA-50	A	2030	2037	2038	2036	2038	2038	2036	2036	2036

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[illegible][illegible]

Figure 3D

[illegible]

Figure 3E

WAA-1	C	14023	14091	14020	14073	14030	14088	14000	14069
WAA-2	C	14140	14141	14140	14139	14139	14134	14139	14139
WAA-3	D	14043	14040	14040	14047	14047	14044	14047	14046
WAA-307	C	17365	17367	17367	17376	17368	17368	17367	
WAA-310	C	22238	22238	22238	22238	22238	22238	22240	
WAA-320	C	39110	39123	39123	39129	39140	39110	39114	
WAA-319	C	33016	33016	33028	33026	33039	33019	33013	
WAA-311	C	37724	37724	37724	37727	37727	37723	37720	
WAA-312	C	44011	44029	44013	44016	44044	44023	44043	
WAA-313	C	47390	47391	47391	47390	47391	47394	47377	
WAA-314	C	51219	51204	51207	51207	51207	51207	51207	
WAA-315	C	56204	56204	56205	56203	56212	56208	56208	
WAA-316	C	59781	59781	59784	59780	59786	59719	59752	
WAA-317	C	64649	64649	64649	64649	64647	64643	64647	
WAA-318	C	74414	74414	74405	74703	74700	74680	74623	
WAA-319	C	76819	76819	76820	76720	76823	76822	76809	
WAA-320	C	80232	80232	80235	80235	80276	80257	80260	
WAA-321	C	83646	83646	83649	83617	83656	83656	83615	
WAA-322	C	86017	86029	86029	86023	86063	86016	86040	
WAA-323	C	89419	89420	89423	89423	89417	89417	89419	
WAA-324	C	89919	89919	89920	89928	89985	89789	89790	
WAA-325	C	110774	110778	110804	110765	110825	110809	110439	
WAA-326	C	116115	116025	116226	116267	116363	116176	116008	
WAA-327	C	120246	120247	120248	120248	120249	120247	120245	
WAA-328	C	140134	140097	140091	140042	140040	140017	140004	
WAA-329	C	160493	160492	160497	160491	160504	160499	160500	
WAA-330	C	160493	160493	160493	160493	160493	160493	160493	
WAA-331	C	177194	177194	177300	177300	177300	177300	177307	
WAA-332	C	181233							
WAA-333	C	190403	190404	190709	190705	190604	190704	190705	
WAA-334	D	20111	20110	20111	20091	20099	20091	20092	
WAA-335	D	20321	20322	20322	20322	20329	20322	20321	
WAA-336	D	20501	20501	20502	20500	20501	20501	20501	
WAA-337	D	20719	20719	20720	20720	20721	20721	20720	
WAA-338	D	21019	21017	21019	21017	21017	21008	21008	
WAA-339	D	21161	21161	21161	21161	21161	21161	21161	
WAA-340	D	22111	22111	22111	22111	22111	22111	22111	
WAA-341	D	22321	22321	22321	22321	22321	22321	22321	
WAA-342	D	22501	22501	22501	22501	22501	22501	22501	
WAA-343	D	22719	22719	22719	22719	22719	22719	22719	
WAA-344	D	22901	22901	22901	22901	22901	22901	22901	
WAA-345	D	23041	23041	23041	23041	23041	23041	23041	
WAA-346	D	24023	24023	24023	24023	24023	24023	24023	
WAA-347	D	24023	24023	24023	24023	24023	24023	24023	
WAA-348	D	24023	24023	24023	24023	24023	24023	24023	
WAA-349	D	24023	24023	24023	24023	24023	24023	24023	
WAA-350	D	24023	24023	24023	24023	24023	24023	24023	
WAA-351	D	24023	24023	24023	24023	24023	24023	24023	
WAA-352	D	24023	24023	24023	24023	24023	24023	24023	
WAA-353	D	24023	24023	24023	24023	24023	24023	24023	
WAA-354	D	24023	24023	24023	24023	24023	24023	24023	

Figure 3F

[illegible]

Figure 3G

[illegible]

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VTA-357 E	0459	0429	0634	0629	0637	0628	0631	0630	0630
VTA-359 E	0705	0648	0781	0783	0852	0853	0845	0857	0846
VTA-359 E	0170	0134	0171	0170	0170	0170	0177	0177	0164
VTA-360 E	0800	0787	0808	0808	0808	0808	0808	0808	0808
VTA-361 E	0452	0476	0476	0484	0482	0482	0470	0468	0477
VTA-362 E	11520	11832	11816	11857	11820	11820	11828	11827	11827
VTA-363 E	11714	11703	11719	11697	11697	11690	11716	11716	11716
VTA-364 E	13844	13864	13864	13864	13864	13864	13864	13864	13864
VTA-365 E	13552	13874	13894	13913	13922	13923	13917	13916	13916
VTA-366 E	13867	13867	13876	13888	13888	13888	13871	13863	13867
VTA-367 E	13916	13916	13916	13916	13916	13916	13916	13916	13916
VTA-368 E	13891	13891	13891	13891	13891	13891	13891	13891	13891
VTA-369 E	17384	17388	17390	17390	17391	17392	17393	17391	17390
VTA-370 E	23289	23289	23289	23289	23289	23289	23289	23289	23289
VTA-371 E	23158	23158	23158	23158	23158	23158	23158	23158	23158
VTA-372 E	23437	23437	23437	23437	23437	23437	23437	23437	23437
VTA-373 E	23432	23432	23432	23432	23432	23432	23432	23432	23432
VTA-374 E	44028	44028	44028	44027	44028	44028	44028	44028	44028
VTA-375 E	01704	01704	01704	01704	01704	01704	01704	01704	01704
VTA-376 E	02769	02769	02769	02769	02769	02769	02769	02769	02769
VTA-377 E	08833	08833	08833	08833	08833	08833	08833	08833	08833
VTA-378 E	70338	70338	70338	70338	70338	70338	70338	70338	70338
VTA-379 E	70384	70384	70384	70384	70384	70384	70384	70384	70384
VTA-380 E	06013	06013	06013	06013	06013	06013	06013	06013	06013
VTA-381 E	00091	00091	00091	00091	00091	00091	00091	00091	00091
VTA-382 E	11047	110158	110854	110854	110776	110776	110776	110776	110776
VTA-383 E	117166	117166	117166	117166	117166	117166	117166	117166	117166
VTA-384 E	132823	132823	132823	132823	132823	132823	132823	132823	132823
VTA-385 E	148032	148032	148032	148032	148032	148032	148032	148032	148032
VTA-386 E	138711	138711	138711	138711	138711	138711	138711	138711	138711
VTA-387 E	108778	108778	108778	108778	108778	108778	108778	108778	108778
VTA-388 E	102870	102870	102870	102870	102870	102870	102870	102870	102870
VTA-389 E	107138	107138	107138	107138	107138	107138	107138	107138	107138
VTA-390 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-391 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-392 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-393 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-394 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-395 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-396 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-397 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-398 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-399 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-400 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-401 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-402 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-403 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-404 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-405 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-406 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-407 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-408 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-409 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-410 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-411 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-412 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-413 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-414 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-415 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-416 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-417 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-418 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-419 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-420 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-421 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-422 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-423 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-424 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-425 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-426 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-427 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-428 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-429 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-430 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-431 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-432 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-433 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-434 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-435 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-436 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-437 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-438 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-439 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-440 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-441 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-442 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-443 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-444 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-445 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-446 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-447 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-448 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-449 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-450 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-451 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-452 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-453 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-454 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-455 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-456 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-457 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-458 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-459 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-460 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-461 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-462 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-463 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-464 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-465 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-466 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-467 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-468 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-469 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-470 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-471 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-472 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-473 E	20711	20711	20711	20711	20711	20711	20711	20711	20711
VTA-474 E	20711	20711	20711	20711	20711	20711</			

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Figure 3J

YAM-451	F	3336	33478	33450	33504	33458	33487	33513	33535
YAM-450	F	43949	43423	43430	43494	43380	43372	43384	43415
YAM-451	F	44857	44888	44329	44758	44688	44675	44679	44682
YAM-452	F	51323	51179	51179	51179	51188	51280	51227	51225
YAM-453	F	59465	59407	59461	59410	59417	59489	59419	59417
YAM-454	F	59549	59526	59544	59438	59438	59438	59432	59430
YAM-455	F	69044	68978	68928	68928	68929	68932	68974	68948
YAM-456	F	73447	73421	73419	73412	73312	73329	73485	73438
YAM-457	F	73257	73208	73345	73345	73134	73273	73246	73294
YAM-458	F	82372	82372	82381	82381	82388	82388	82387	82332
YAM-459	F	86475	86279	86279	86279	86284	86284	86278	86288
YAM-479	F	94442	94791	94428	94785	94785	94743	94729	94655
YAM-471	F	100881	100435	100480	100214	100295	100284	100284	100284
YAM-472	F	114742	113705	113707	113705	113692	113704	113692	113692
YAM-473	F	123204	123278	123009	123440	123785	123785	123785	123785
YAM-475	F	144237	144729	144729	144880	144882	144882	144919	144919
YAM-473	F	162388	162388	161482	160793	160845	160845	160845	160270
YAM-479	A								
YAM-477	A				3584				
YAM-478	A				2614				
YAM-479	A								
YAM-480	A			3423					
YAM-481	A								
YAM-482	A								
YAM-483	A								
YAM-484	A		3857	3886	3296			3422	3425
YAM-485	A								
YAM-486	A					2367			
YAM-487	A								
YAM-488	A			2573					
YAM-489	A								2679
YAM-490	A					2731			
YAM-491	A				2575				
YAM-492	A	2991	2971			2362			
YAM-493	A								
YAM-494	A				6033				
YAM-495	A								
YAM-496	A					3047			
YAM-497	A						3204		
YAM-498	A						3261		
YAM-499	A						3289		
YAM-500	A						3365		
YAM-501	A						3400		
YAM-502	A						3511		
YAM-503	A						3723		
YAM-504	A						4181		
YAM-505	A						4181		
YAM-506	A				4314		4214		
YAM-507	A						4268		
YAM-508	A	4528				4539	4234		
YAM-509	A						4279		
YAM-510	A								

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Figure 3k

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Figure 3L

WMA-001 B	2549	2549	2539	3457	3457		
WMA-002 B			1973	3239	3239		
WMA-003 B	2579	2579	3182	3276	3276		
WMA-004 B	3184	3184	3274	3274	3274		
WMA-005 B			3274	3274	3274		
WMA-006 B	3269	3269	3277	3299	3299		
WMA-007 B	3379	3379		3379	3379		
WMA-008 B				3499	3499		
WMA-009 B				3505	3505		
WMA-010 B			2771	3565	3565		
WMA-011 B				3779	3779		
WMA-012 B	3788	3772		3843	3843		
WMA-013 B				3943	3943		
WMA-014 B			4491	4009	4009		
WMA-015 B			4149	4082	4082		4082
WMA-016 B	4252	4491	4491	4147	4132		
WMA-017 B	4148	4443	4447	4147	4132		
WMA-018 B	4447	4447	4732	4447	4447		
WMA-019 B	4780	4784	4784	4783	4783		4672
WMA-020 B		4999	5443				4677
WMA-021 B	5442	5443	5388	5442	5442		
WMA-022 B	5957	5959	6184	5957	5957		
WMA-023 B	6187	6187	6609	6184	6184		
WMA-024 B							
WMA-025 B				10585	10585		16255
WMA-026 B		10267		17922	17922		
WMA-027 B				53722	53722		
WMA-028 B		53738	53888	53827	53827		17446
WMA-029 B	53913	53843			155465		11908
WMA-030 B					171835		
WMA-031 B				2021	2021		
WMA-032 B				2138	2138		
WMA-033 B	2138	2138	2286		2225		
WMA-034 B			2419				
WMA-035 B			3438				
WMA-036 B							
WMA-037 B	3438	3437	3574		3410		
WMA-038 B					3410		
WMA-039 B					3410		
WMA-040 B					3410		
WMA-041 B					3410		
WMA-042 B					3410		
WMA-043 B					3410		
WMA-044 B					3410		
WMA-045 B					3410		
WMA-046 B					3410		
WMA-047 B					3410		
WMA-048 B					3410		
WMA-049 B					3410		
WMA-050 B					3410		
WMA-051 B					3410		
WMA-052 B					3410		
WMA-053 B					3410		
WMA-054 B					3410		
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WMA-068 B					3410		
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WMA-070 B					3410		
WMA-071 B					3410		
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WMA-097 B					3410		
WMA-098 B					3410		
WMA-099 B					3410		
WMA-100 B					3410		

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Figure 3M

WMA-012 C	3372	3379	3419	3373	3364		
WMA-013 C			3854		3576		
WMA-014 C							
WMA-015 C			3722				3716
WMA-016 C			3773		3769		
WMA-017 C	3779	3779	3781	3776			
WMA-018 C			3803				
WMA-019 C			3829				
WMA-020 C			3869				
WMA-021 C			3879	3852	3861		3858
WMA-022 C	3866		4010		4010		3953
WMA-023 C			4118	4021	4010		3948
WMA-024 C	4012		4185		4183		4158
WMA-025 C		4186	4267		4183		4158
WMA-026 C			4302				
WMA-027 C			4433				
WMA-028 C							
WMA-029 C							
WMA-030 C							
WMA-031 C							
WMA-032 C	5097	5102	5214	5214	5204		
WMA-033 C			5285	5285	5285		
WMA-034 C			5357	5357	5357		
WMA-035 C			5388	5388	5388		
WMA-036 C	54015	53879	53888	53888	53888		53888
WMA-037 C			54094	54094	54094		53888
WMA-038 C			54181	54181	54181		53888
WMA-039 C							
WMA-040 C							
WMA-041 C							
WMA-042 C							
WMA-043 C							
WMA-044 C							
WMA-045 C							
WMA-046 C							
WMA-047 C							
WMA-048 C							
WMA-049 C							
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WMA-099 C							
WMA-100 C							

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Figure 3 N

[illegible]

Figure 30.

WM714 F			04150	04672			04125	04108	
WM715 F						101041	04712	04790	
WM716 F							102354	102375	
WM717 F								100000	

Figure 4A

Rank	Normal vs Cancer	Adeno vs Normal	Squamous vs Normal	Small Cell vs Normal	Non-small Cell vs Normal	Large Cell vs Normal	Adeno vs Squamous	Adeno vs Small Cell	Squamous vs Small Cell
1	WM-61	WM-447	WM-447	WM-70	WM-341	WM-18	WM-42	WM-437	WM-278
2	WM-447	WM-452	WM-61	WM-708	WM-342	WM-28	WM-15	WM-72	WM-277
3	WM-446	WM-61	WM-277	WM-329	WM-343	WM-28	WM-12	WM-329	WM-352
4	WM-133	WM-446	WM-446	WM-447	WM-48	WM-134	WM-385	WM-78	WM-252
5	WM-119	WM-290	WM-133	WM-61	WM-340	WM-447	WM-347	WM-70	WM-353
6	WM-278	WM-353	WM-134	WM-252	WM-349	WM-277	WM-134	WM-73	WM-347
7	WM-134	WM-133	WM-353	WM-252	WM-47	WM-353	WM-128	WM-44	WM-254
8	WM-353	WM-341	WM-352	WM-446	WM-329	WM-448	WM-119	WM-17	WM-43
9	WM-252	WM-285	WM-276	WM-456	WM-389	WM-448	WM-119	WM-43	WM-252
10	WM-442	WM-358	WM-705	WM-134	WM-059	WM-221	WM-151	WM-63	WM-431
11	WM-120	WM-282	WM-203	WM-134	WM-447	WM-448	WM-280	WM-42	WM-431
12	WM-290	WM-353	WM-486	WM-548	WM-022	WM-437	WM-353	WM-63	WM-513
13	WM-45	WM-810	WM-359	WM-439	WM-184	WM-250	WM-61	WM-412	WM-446
14	WM-277	WM-222	WM-45	WM-45	WM-057	WM-447	WM-211	WM-453	WM-447
15	WM-70	WM-130	WM-70	WM-458	WM-458	WM-328	WM-117	WM-448	WM-355
16	WM-353	WM-134	WM-341	WM-473	WM-430	WM-426	WM-352	WM-313	WM-133
17	WM-17	WM-278	WM-429	WM-343	WM-283	WM-183	WM-130	WM-458	WM-245
18	WM-473	WM-428	WM-347	WM-438	WM-207	WM-180	WM-414	WM-66	WM-62
19	WM-47	WM-277	WM-341	WM-341	WM-435	WM-438	WM-277	WM-79	WM-66
20	WM-329	WM-29	WM-47	WM-340	WM-394	WM-307	WM-141	WM-348	WM-236
21	WM-278	WM-119	WM-431	WM-353	WM-61	WM-458	WM-61	WM-380	WM-243
22	WM-279	WM-340	WM-62	WM-339	WM-167	WM-30	WM-133	WM-180	WM-138
23	WM-62	WM-48	WM-473	WM-457	WM-302	WM-17	WM-417	WM-418	WM-62
24	WM-358	WM-358	WM-334	WM-66	WM-285	WM-545	WM-353	WM-63	WM-580
25	WM-438	WM-430	WM-438	WM-308	WM-850	WM-47	WM-338	WM-257	WM-134
26	WM-425	WM-47	WM-632	WM-72	WM-203	WM-101	WM-63	WM-138	WM-640
27	WM-284	WM-343	WM-282	WM-257	WM-119	WM-147	WM-142	WM-47	WM-258
28	WM-287	WM-17	WM-359	WM-62	WM-252	WM-438	WM-448	WM-252	WM-209
29	WM-420	WM-583	WM-290	WM-425	WM-635	WM-280	WM-156	WM-252	WM-111
30	WM-482	WM-70	WM-378	WM-65	WM-353	WM-218	WM-111	WM-60	WM-66
31	WM-431	WM-708	WM-430	WM-73	WM-429	WM-285	WM-445	WM-68	WM-347
32	WM-453	WM-348	WM-673	WM-436	WM-11	WM-052	WM-455	WM-323	WM-157
33	WM-29	WM-468	WM-340	WM-384	WM-208	WM-051	WM-276	WM-402	WM-342
34	WM-340	WM-648	WM-65	WM-65	WM-451	WM-385	WM-444	WM-411	WM-558
35	WM-19	WM-384	WM-485	WM-485	WM-473	WM-63	WM-181	WM-400	WM-63
36	WM-359	WM-338	WM-445	WM-310	WM-220	WM-418	WM-35	WM-78	WM-229
37	WM-63	WM-284	WM-128	WM-277	WM-485	WM-430	WM-258	WM-417	WM-284
38	WM-436	WM-339	WM-420	WM-70	WM-338	WM-468	WM-458	WM-467	WM-274

Figure 4B

39	WM-450	WM-473	WM-480	WM-207	WM-71	WM-714	WM-39	WM-25	WM-370
40	WM-455	WM-359	WM-509	WM-278	WM-285	WM-046	WM-62	WM-410	WM-301
41	WM-208	WM-38	WM-270	WM-690	WM-70	WM-189	WM-17	WM-420	WM-440
42	WM-343	WM-203	WM-342	WM-385	WM-345	WM-302	WM-253	WM-184	WM-74
43	WM-341	WM-825	WM-471	WM-472	WM-673	WM-667	WM-60	WM-184	WM-74
44	WM-330	WM-86	WM-474	WM-420	WM-448	WM-578	WM-412	WM-68	WM-467
45	WM-55	WM-45	WM-120	WM-147	WM-120	WM-131	WM-89	WM-391	WM-227
46	WM-68	WM-850	WM-20	WM-66	WM-257	WM-708	WM-74	WM-340	WM-262
47	WM-48	WM-307	WM-287	WM-680	WM-468	WM-338	WM-457	WM-425	WM-225
48	WM-38	WM-278	WM-63	WM-357	WM-347	WM-353	WM-431	WM-188	WM-225
49	WM-138	WM-342	WM-154	WM-129	WM-123	WM-65	WM-340	WM-312	WM-304
50	WM-310	WM-429	WM-128	WM-270	WM-33	WM-466	WM-48	WM-132	WM-37

10 20 30 40 50 60 70 80 90 100
 RSFFSFLCEAFDGDARDNWRAYSMDREANYIGSDKYFHARGNYDAAKRGPGGVWAAEASDARENIQRFHGHGAEDSLADQAANEWGRSGKDPNHFRLPAGLPEKY

2803
 SAA 42-67 (2802.1)
 3168
 SAA 69-97 (3167.3)
 3277
 SAA 39-68 (3276.6)
 3552
 SAA 38-70 (3552)
 3897
 SAA 64-98 (3897.2)
 4300
 SAA 54-93 (4302.5)
 4490
 SAA 53-93 (4489)
 4655
 SAA 5-44 (4655.0)

5927
 SAA 32-85 (5925.3)
 6874
 SAA 26-88 (6873.3)
 7776
 SAA 1-68 (7774.6)
 7941
 SAA 18-88 (7939.5)
 8152
 SAA 25-98 (8150)
 8952
 SAA 6-85 (8950)
 9233
 SAA 16-97 (9235)

10300
 SAA 6-97 (10299.1)
 10866
 SAA 4-101 (10871.8)
 10851
 SAA 5-102 (10853.7)

Figure 5

Figure 6

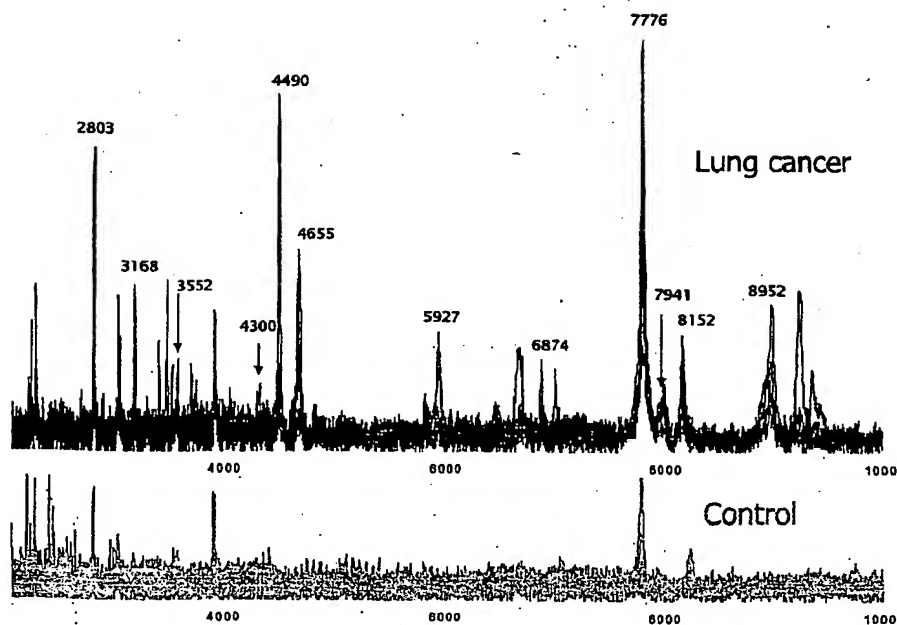
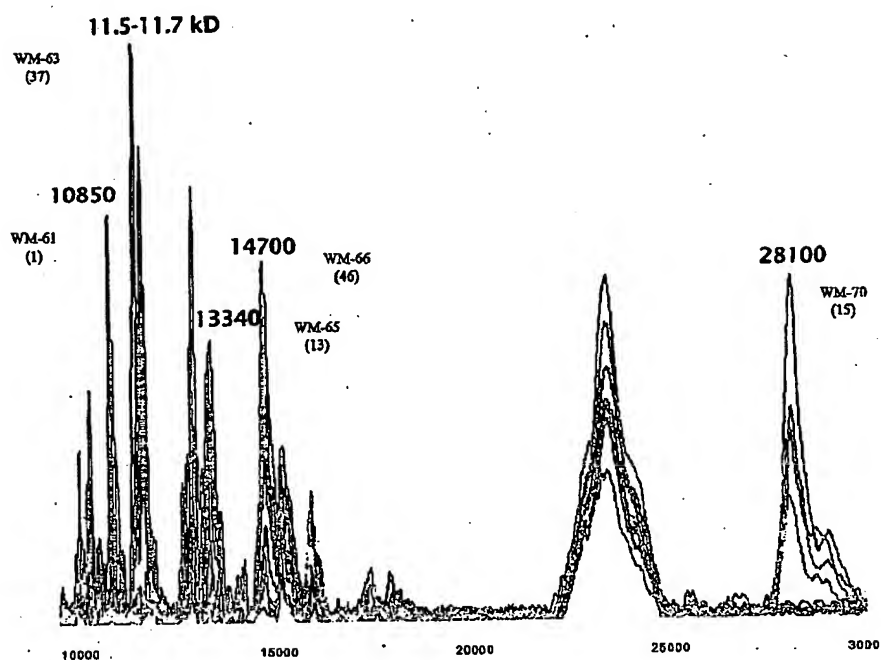


FIGURE 7
Protein Profile of Selected Samples Q Fraction 1 WCX2

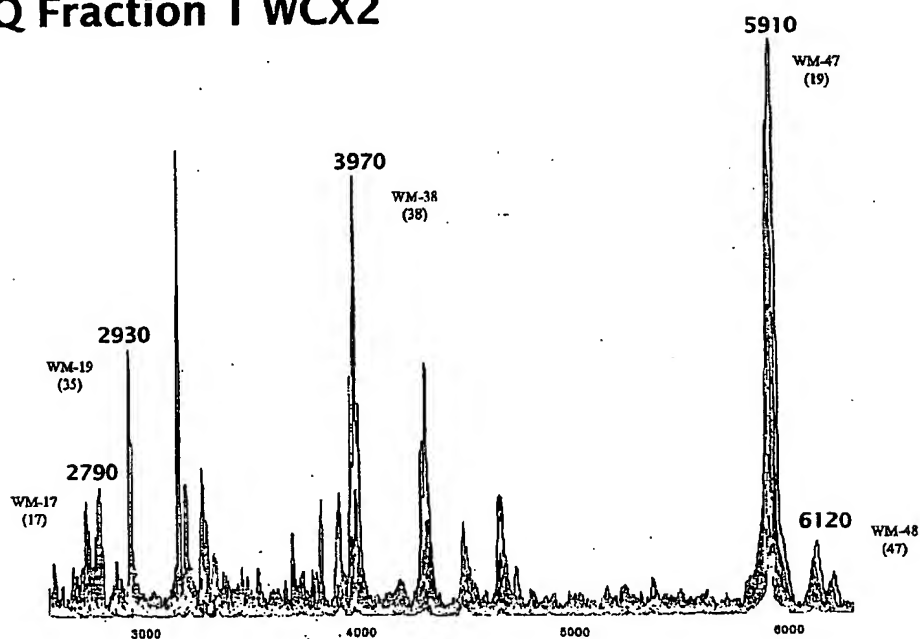


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Figure 8
Protein Profile of Selected Samples
Q Fraction 1 WCX2

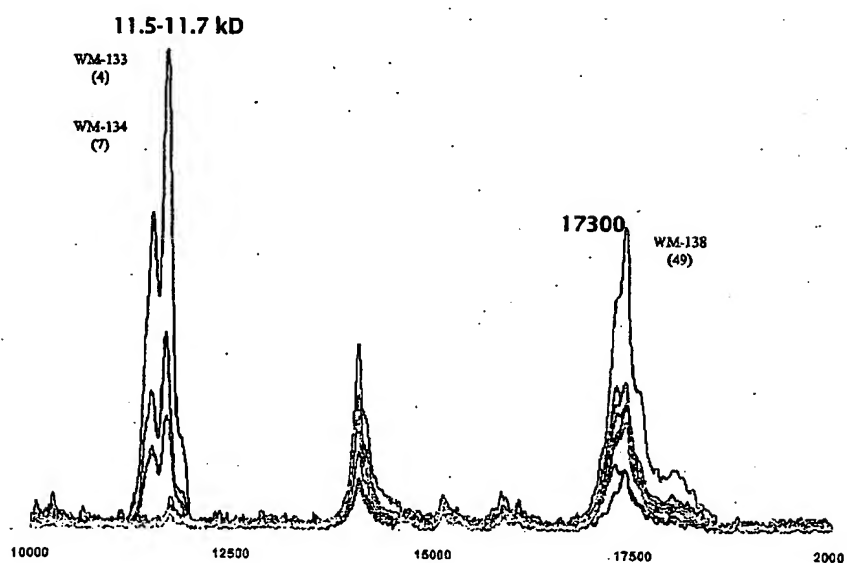


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Figure 9
Protein Profile of Selected Samples
Q Fraction 2 WCX2

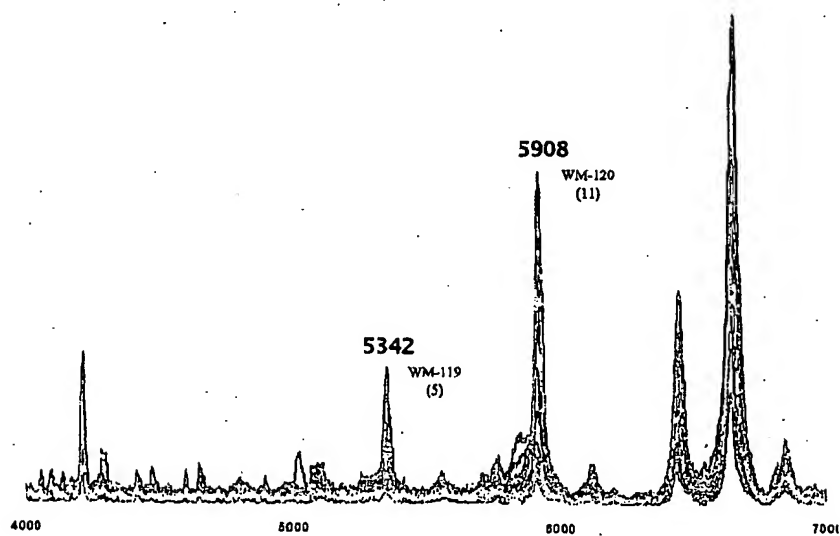


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Figure 10
Protein Profile of Selected Samples
Q Fraction 2 WCX2

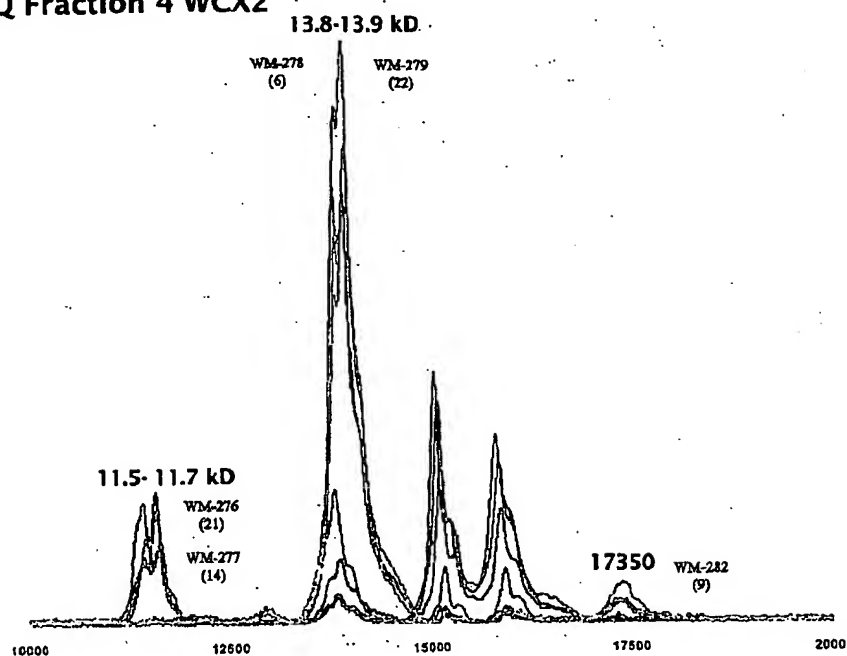


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Figure 11
Protein Profile of Selected Samples
Q Fraction 4 WCX2

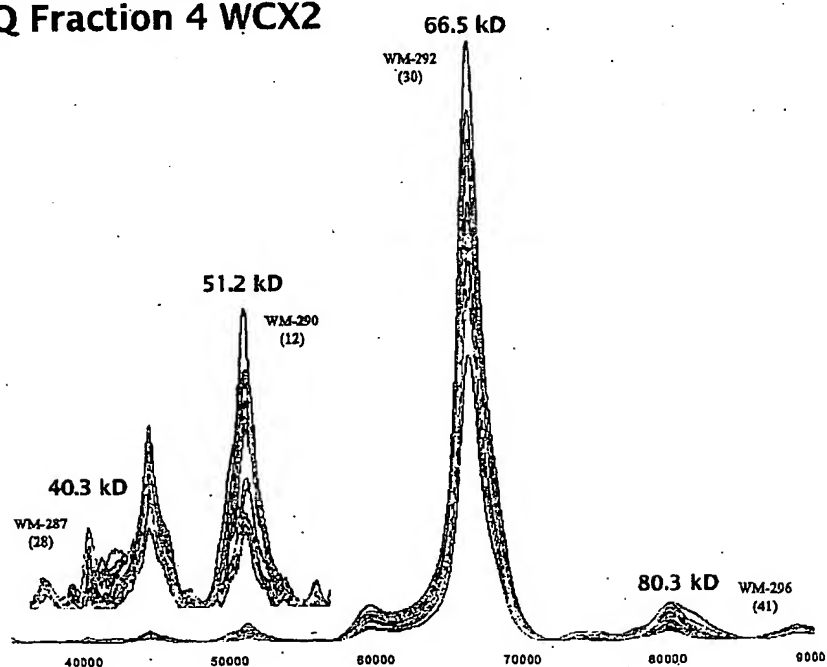


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Figure 12
Protein Profile of Selected Samples
Q Fraction 4 WCX2

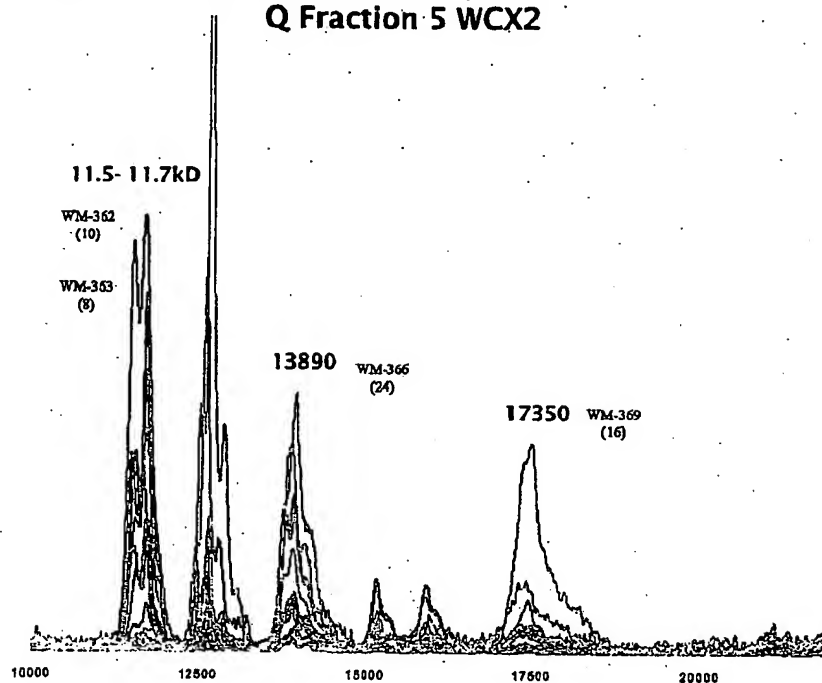


W0 2004061410

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Figure 13
Protein Profile of Selected Samples
Q Fraction 5 WCX2

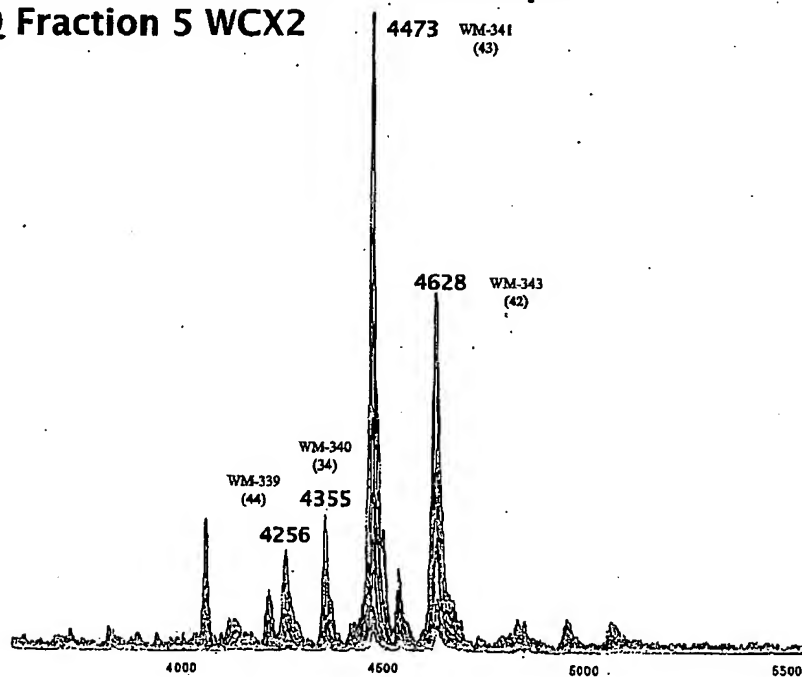


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Figure 14
Protein Profile of Selected Samples
Q Fraction 5 WCX2

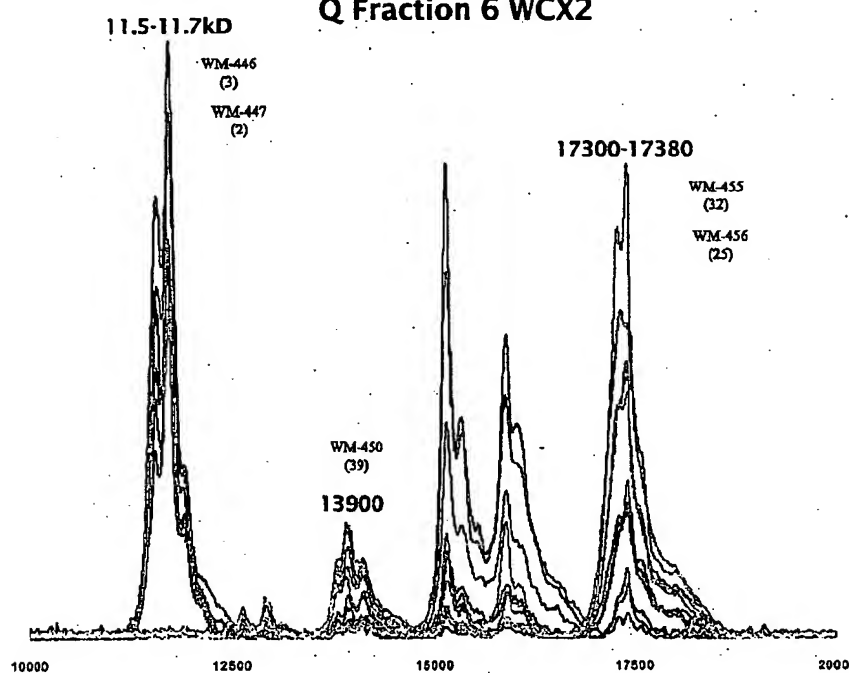


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Figure 15
Protein Profile of Selected Samples
Q Fraction 6 WCX2

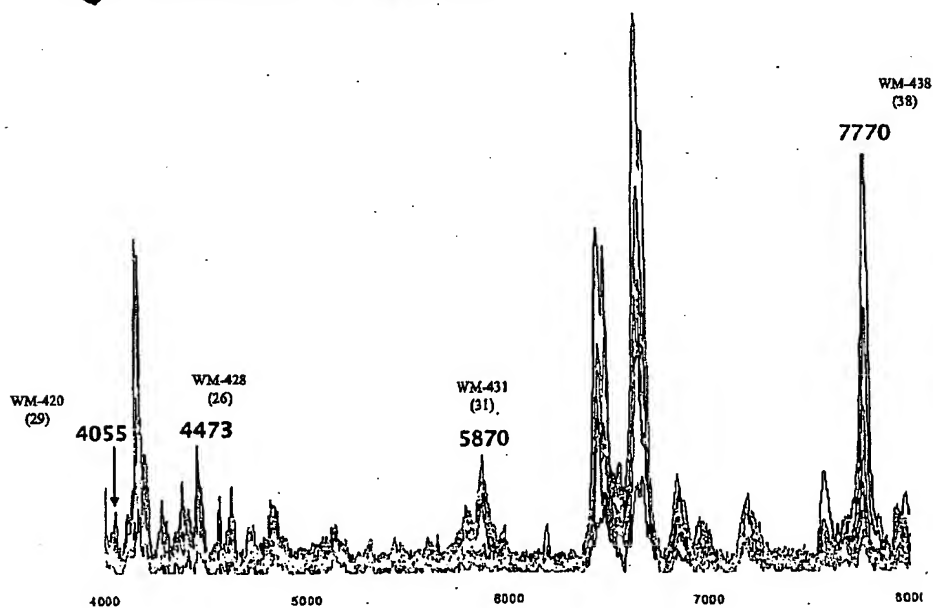


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Figure 16
Protein Profile of Selected Samples
Q Fraction 6 WCX2

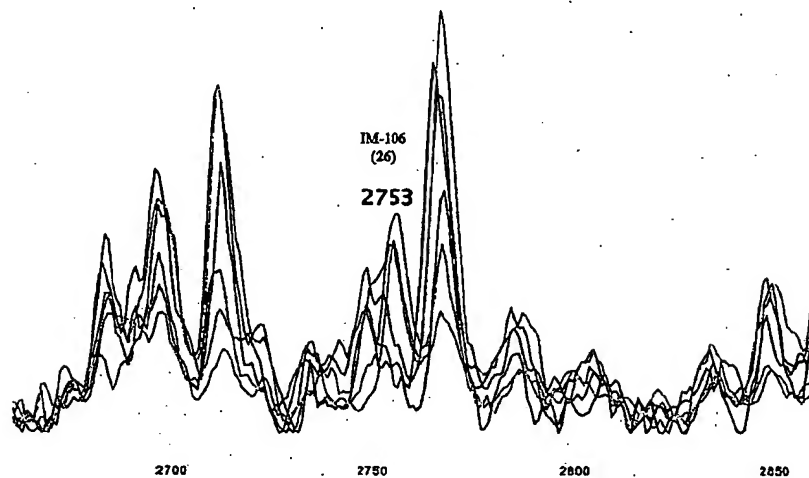


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Figure 17
Protein Profile of Selected Samples
Q Fraction 2 IMAC-Cu(II)

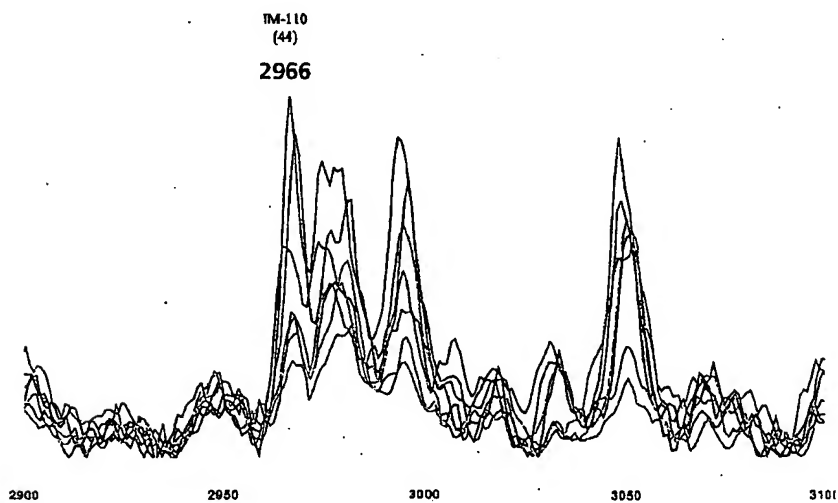


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Figure 18
Protein Profile of Selected Samples
Q Fraction 2 IMAC-Cu(II)

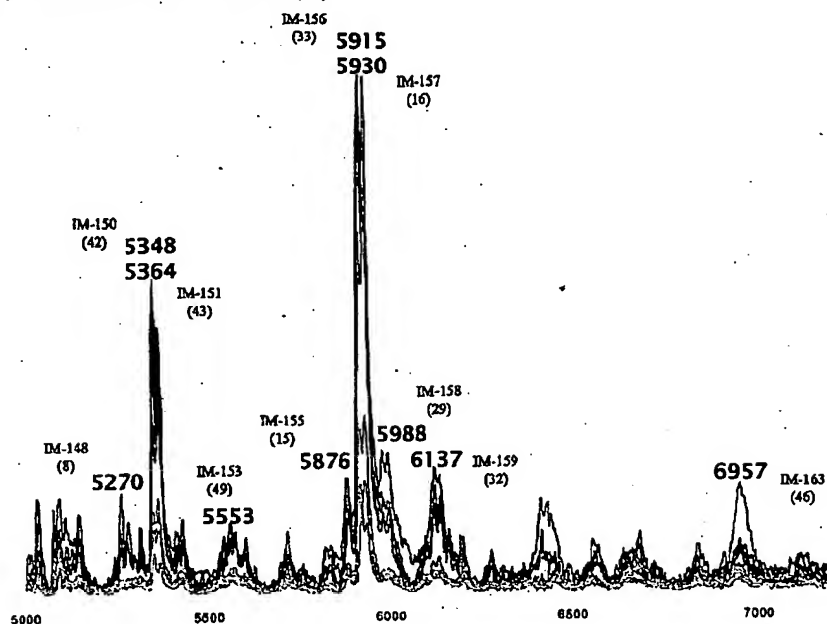


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Figure 19
Protein Profile of Selected Samples
Q Fraction 2 IMAC-Cu(II)

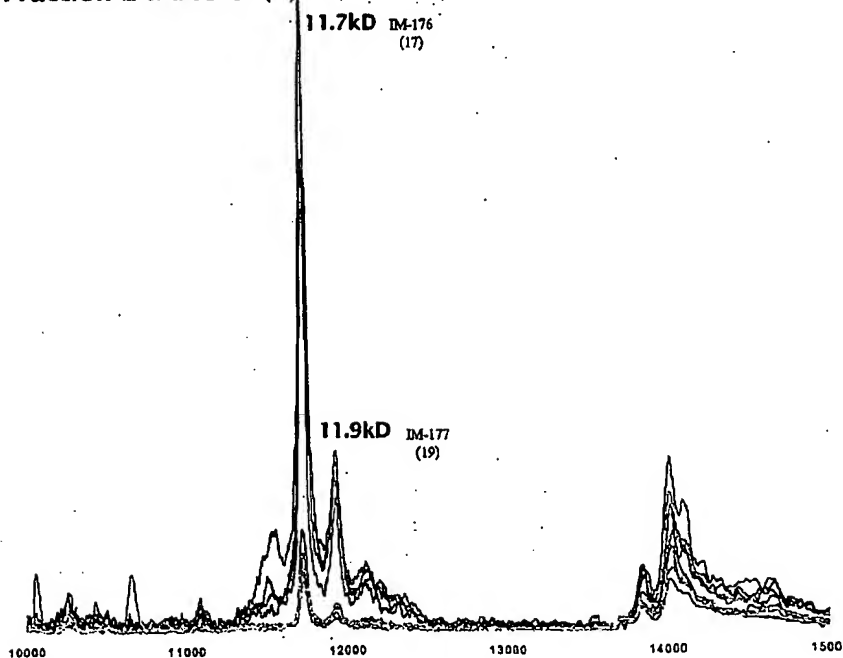


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Figure 20
Protein Profile of Selected Samples
Q Fraction 2 IMAC-Cu(II)



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Figure 21

Protein Profile of Selected Samples Q Fraction 3 IMAC-Cu(II)

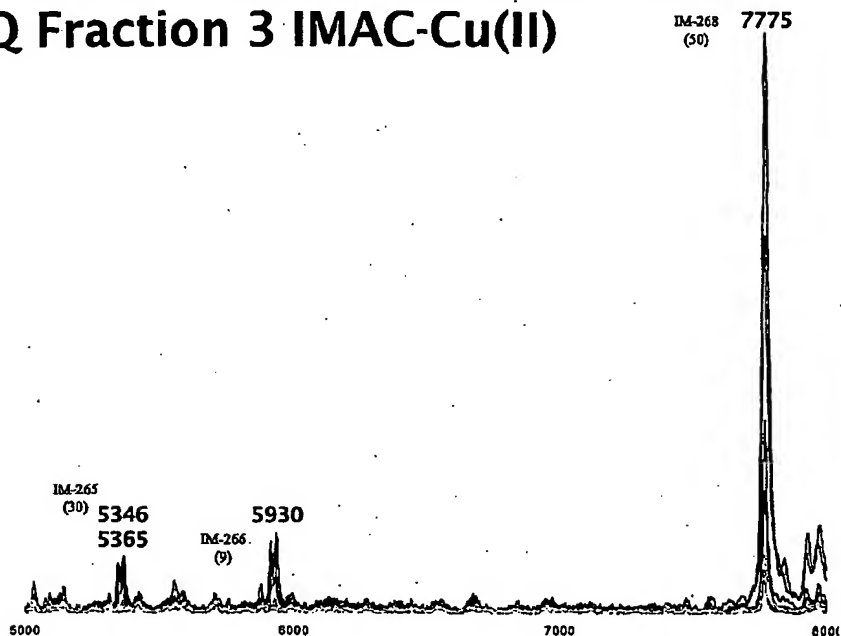


Figure 22

Protein Profile of Selected Samples Q Fraction 3 IMAC-Cu(II)

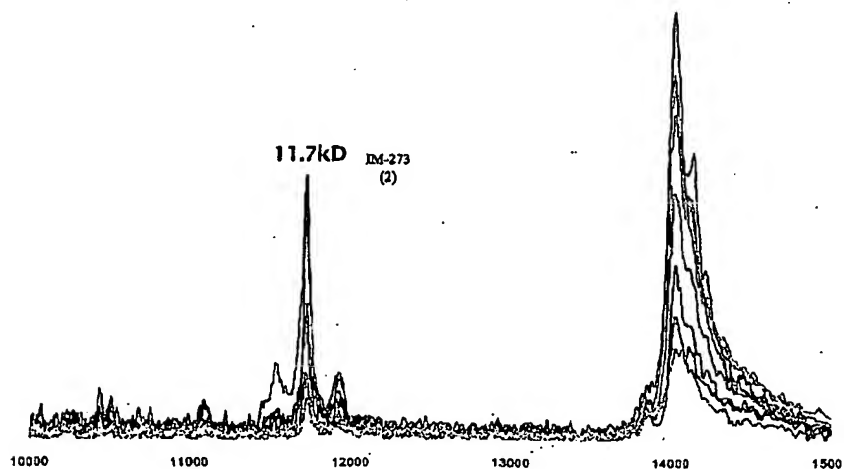
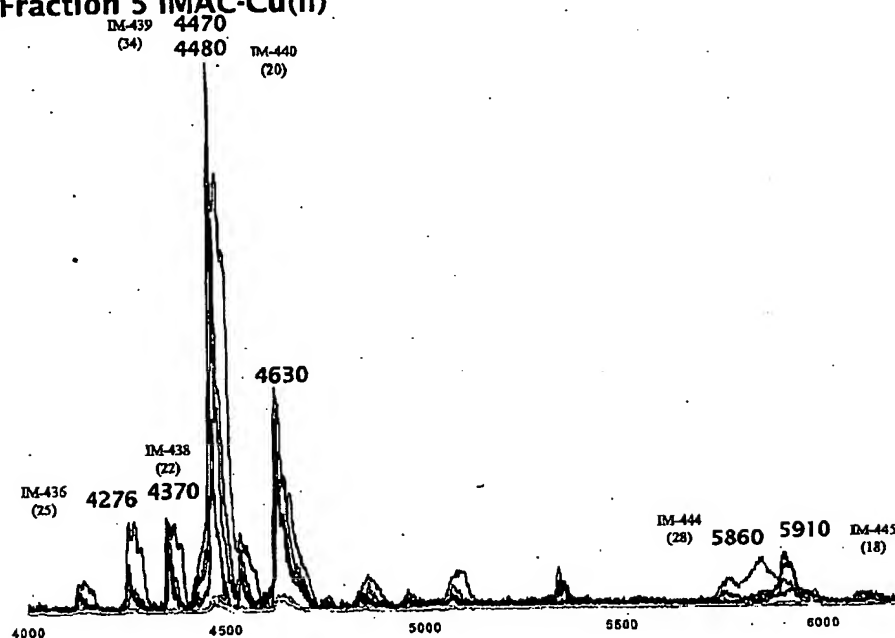


Figure 23
Protein Profile of Selected Samples
Q Fraction 5 IMAC-Cu(II)

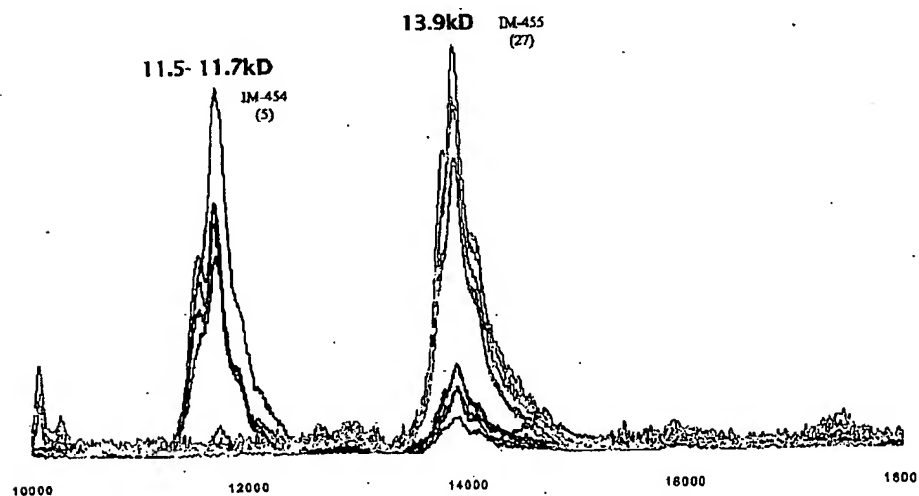


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Figure 24
Protein Profile of Selected Samples
Q Fraction 5 IMAC-Cu(II)

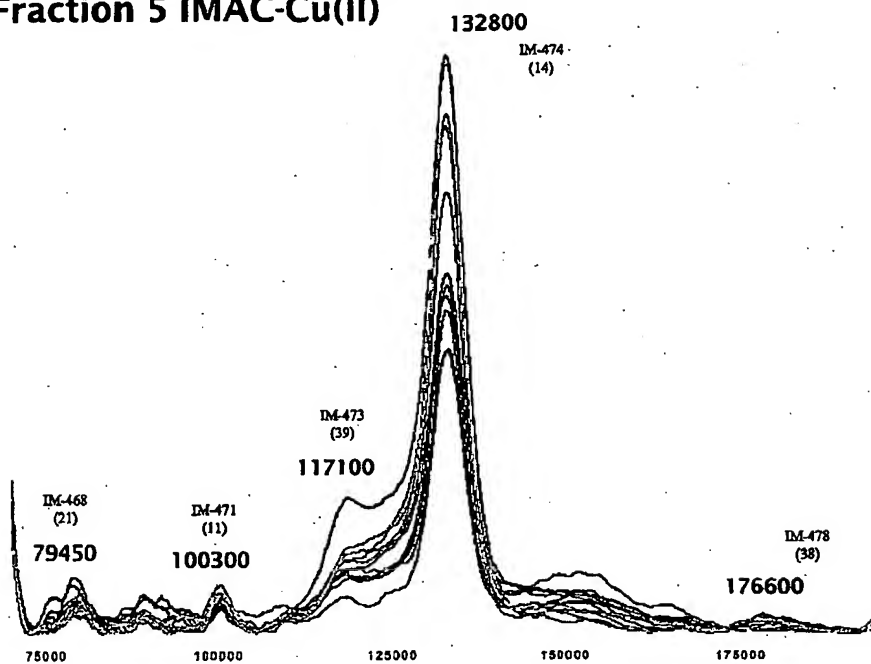


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Figure 25
Protein Profile of Selected Samples
Q Fraction 5 IMAC-Cu(II)

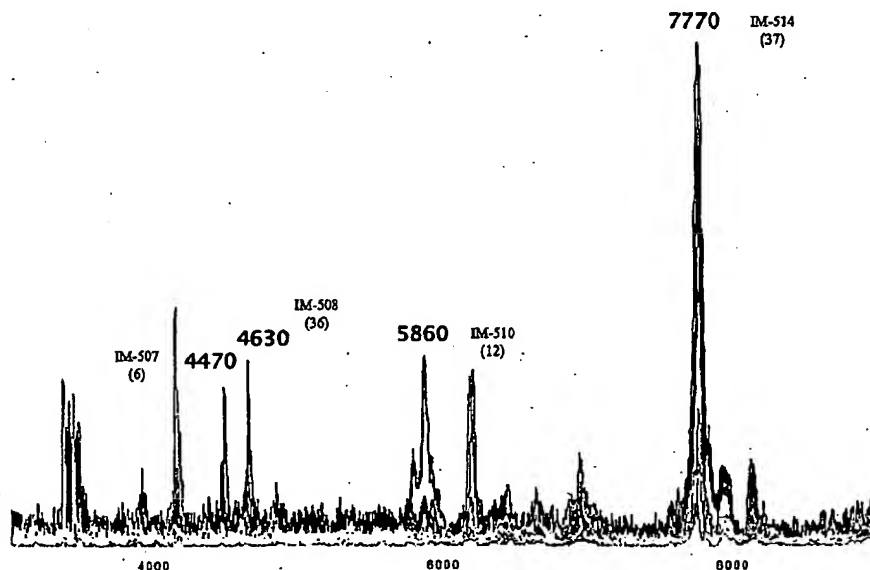


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Figure 26
Protein Profile of Selected Samples
Q Fraction 6 IMAC-Cu(II)

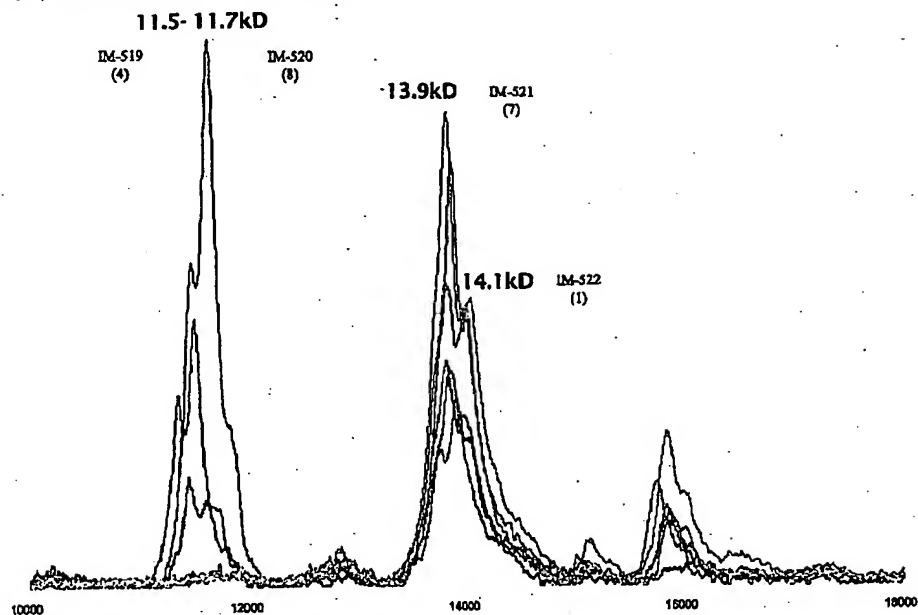


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Figure 27
Protein Profile of Selected Samples
Q Fraction 6 IMAC-Cu(II)

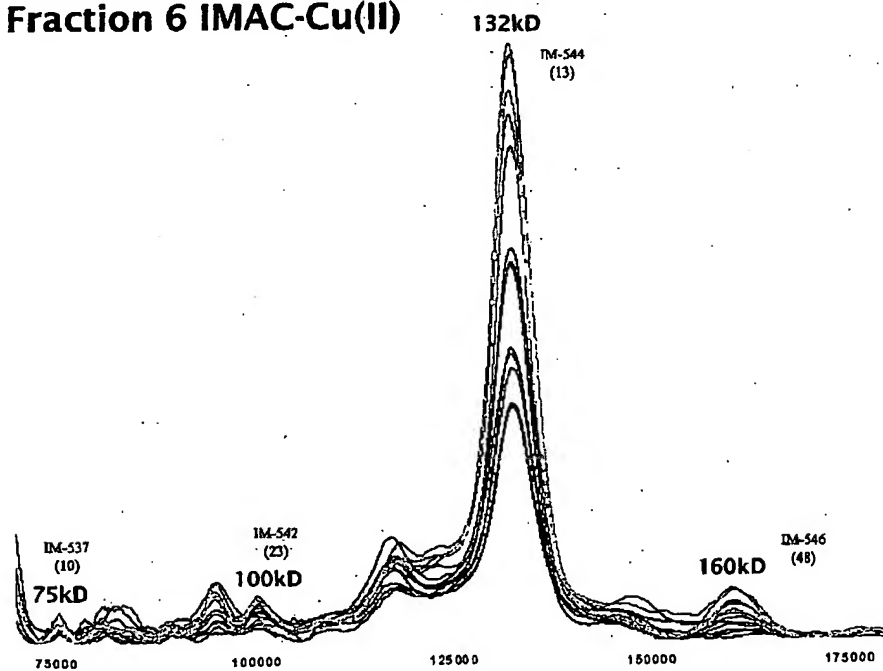


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Figure 28
Protein Profile of Selected Samples
Q Fraction 6 IMAC-Cu(II)



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